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Review Article

CYANOBACTERIA - AS POTENTIAL BIOFERTILIZER

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ABSTRACT
Cyanobacteria are one of the major components of the nitrogen fixing biomass in paddy fields. and provides a potential source of nitrogen fixation at no cost. Due to the important characteristic of nitrogen fixation, cyanobacteria have a unique potential to contribute to enhance productivity in a variety of agricultural and ecological situations. Cyanobacteria play an important role to build-up soil fertility consequently increasing the yield. Biofertilizer being essential components of organic farming play vital role in maintaining long term soil fertility and sustainability by fixing atmospheric dinitrogen (N=N), mobilizing fixed macro and micro nutrients or convert insoluble phosphorus in the soil into forms available to plants, thereby increases their efficiency and availability. The blue green algae (cyanobacteria) are capable of fixing the atmospheric nitrogen and convert it into an available form of ammonium required for plant growth. Dominant nitrogen-fixer blue-green algae are *Anabaena, Nostoc, Aulosira, Calothrix, Plectonema* etc. Blue-green algae have the abilities of photosynthesis as well as biological nitrogen fixation. Cyanobacteria are one of the major components of the nitrogen fixing biomass in paddy fields. The agricultural importance of cyanobacteria in rice cultivation is directly related with their ability to fix nitrogen and other positive effects for plants and soil. Bio-fertilizers are eco-friendly and have been proved to be effective and economical alternate of chemical fertilizers with lesser in put of capital and energy.

Key Words: *Fertilizer, Cyanobacteria, Nitrogen Fixation, Nutrient, Rice Field*

INTRODUCTION
Biofertilizer is defined as a substance, contains living microorganisms which colonizes the rhizosphere or the interior of the plant and promotes growth by increasing the supply or availability of primary nutrient and/or growth stimulus to the target crop, when applied to seed, plant surfaces, or soil (Vessey, 2003). The biofertilizer are natural fertilizes which are living microbial inoculants of bacteria, algae, fungi alone or in combination and they augment the availability of nutrients to the plants. Bio-fertilizers containing beneficial bacteria and fungi improve soil chemical and biological characteristics, phosphate solutions and agricultural production (El-Habbasha *et al*., 2007; Yosefi *et al*., 2011). The use of biofertilizer, in preference to chemical fertilizers, offers economic and ecological benefits by way of soil health and fertility to farmers. Biofertilizers add nutrients through the natural processes of Nitrogen fixation, solubilizing phosphorus and stimulating plant growth through the synthesis of growth promoting substances. Biofertilizers can be expected to reduce the use of chemical fertilizers and pesticides. The microorganisms (Azotobacter, Blue green algae, Rhizobium Azospirillum) in biofertilizer restore the soil's natural nutrient cycle and build soil organic matter. Biofertilizer contains microorganisms which promote the adequate supply of nutrients to the host plants and ensure their proper development of growth and regulation in their physiology. Living microorganisms are used in the preparation of Biofertilizer, only those microorganisms are used which have specific functions to enhance plant growth and reproduction. Microorganism converts complex nutrients into simple nutrients for the availability of the plants. Crop yield can be increased by 20-30% if biofertilizer are used properly. Biofertilizer can also

protect plants from soil born diseases to a certain degree. The need for the use of biofertilizer has arisen, primarily for two reasons. First, because increase in the use of fertilizers leads to increased crop productivity, second, because increased usage of chemical fertilizer leads to damage in soil texture and raises other environmental problems. Therefore, the use of biofertilizer is both economical and environment friendly.

Organisms Used As Biofertilizer

Microbiological fertilizers are important to environment friendly sustainable agricultural practices (Bloemberg *et al.*, 2000). The Biofertilizer includes mainly the nitrogen fixing, phosphate solubilizing and plant growth promoting microorganisms (Goel *et al.*, 1999). Among biofertilizer benefiting the crop production are Azotobacter, Azospirillium, Blue Green Algae, Azolla, P-solubilizing micro organisms, Mycorrhizae and Sinorhizobium (Hegde *et al.*, 1999). There are different types of microorganisms which are used as the biofertilizer. Some are capable of nitrogen fixation such as Azotobacter, Blue green algae, Rhizobium and Azospirillum. Rhizobium is used to increase the capacity of nitrogen fixation in the leguminous plants. Azotobacter are used as biofertilizers for the development of various vegetable plants such as mustard, maize, wheat, cotton etc. Azospirillum is applied in the millets, sorghum, sugarcane, maize and wheat field. Blue green algae such as *Nostoc, Tolypothrix, Anabaena,* and *Aulosira* fix atmospheric nitrogen and enrich the soil fertility (Table.1).

Table 1: Important microorganisms constituting Biofertilizer and their application for variety of crops

Microorganisms use as biofertilizer	Nutrient fixed (Kg/ha/year)	Beneficiary Crops
Rhizobium	50 to 300 kg N / ha	Groundnut, Soybean, Redgram, Green-gram, Black-gram, Lentil, Cowpea, Bengal-gram and Fodder legumes
Azotobacter	0.026 to 20 kg N / ha	Cotton, Vegetables, Mulberry, Plantation Crop, Rice, Wheat, Barley, Ragi, Jowar, Mustard, Safflower, Niger, Sunflower, Tobacco, Fruit , Spices, Condiment, Ornamental Flower
Azospirillum	10-20 kg N /ha	Sugarcane, Vegetables, Maize, Pearl millet, Rice, Wheat, Fodders, Oil seeds, Fruit and Flower
Blue Green Algae	25 kg N /ha	Rice, banana
Azolla	900 kg N /ha	Rice
Phosphate solubilizing bacteria and fungi	Solubilize about 50-60%of them fixed phosphorus in the soil	All Crops (non specific)

Cyanobacteria Used As Biofertilizer

Cyanobacteria play an important role in maintenance and build up of soil fertility, consequently increasing rice growth and yield as a natural biofertilizer (Song *et al.,* 2005). The acts of these algae include: (1) Increase in soil pores with having filamentous structure and production of adhesive substances. (2) Excretion of growth-promoting substances such as hormones (auxin, gibberellin), vitamins, amino acids (Roger and Reynaud 1982, Rodriguez *et al.,* 2006). (3) Increase in water holding capacity through their jelly structure (Roger and Reynaud 1982). (4) Increase in soil biomass after their death and decomposition (Saadatnia and Riahi, 2009) (5) Decrease in soil salinity (Saadatnia and Riahi, 2009) (6) Preventing weeds growth (Saadatnia and Riahi , 2009) (7) Increase in soil phosphate by excretion of organic acids (Wilson 2006). Beneficial effects of cyanobacterial inoculation were also reported on a number of other crops such as barley, oats, tomato, radish, cotton, sugarcane, maize, chilli and lettuce (Thajuddin and Subramanian 2005). Microalgae (including blue-green algae-BGA or cyanobacteria) are diverse groups of photoautotrophic microorganisms comprising of a large, heterogeneous, and polyphyletic assemblage of relatively simple plants. Most microalgae usually occur in water, be it freshwater, marine, or brackish. They can also be found in extreme environments e.g. hot springs (Anderson, 2005).

Cyanobacteria or Blue green algae (BGA) are a group of microorganism that can fix the atmospheric nitrogen. BGA can adapt to various soil types and environment which has made it cosmopolitan in distribution. Efficient nitrogen fixing strain like *Nostoc linkia, Anabaena variabilis, Aulosira fertilisima, Calothrix sp., Tolypothrix sp., and Scytonema sp.* were identified from various agro-ecological regions and utilized for rice production (Prasad and Prasad, 2001). After water, nitrogen is the second limiting factor for plant growth in many fields and deficiency of this element is met by fertilizers (Malik *et al.,* 2001). Cyanobacteria play an important role in maintenance and build-up of soil fertility, consequently increasing rice growth and yield as a natural biofertilizer (Song *et al.,* 2005). Blue green algae (BGA) are photosynthetic nitrogen fixers and are free living. They are found in abundance in India. They too add growth promoting substances including vitamin $B_{12,}$ improve the soil's aeration and water holding capacity and add to biomass when decomposed after life cycle. *Azolla* is an aquatic fern found in small and shallow water bodies and in rice fields. It has symbiotic relation with BGA and can help rice or other crops through dual cropping or green manuring of soil. They manufacture their food by photosynthesis, as they have chloroplasts. Hence, they can live independently. Heterocystous nitrogen-fixing blue-green algae consist of filaments containing two types of cells: the heterocysts, responsible for ammonia synthesis, and vegetative cells, which exhibit normal photosynthesis and reproductive growth. Cyanobacteria are capable of abating various kinds of pollutants and have advantages as potential biodegrading organisms (Subramanian and Uma, 1996). As these organisms have simple growth requirements, they could be attractive host for production of valuable organic products. Malliga *et al.,* (1996) have reported that *Anabaena azollae* while being used as a biofertilizer exhibited lignolysis and released phenolic compounds which induced profuse sporulation of the organism. This report gives the usefulness of coir waste as carrier for cyanobacterial biofertilizer with supporting enzyme studies on lignin degrading ability of cyanobacteria and use of lignocellulosic coir waste as an excellent and inexpensive carrier for cyanobacterial biofertilizer. BGA like *Anabaena* and *Nostoc* are found to live on soil, rocks. They have potentiality to fix large amount of atmospheric nitrogen (up to 20 - 25 kg/ha). Blue green algae belonging to genera *Nostoc, Anabaena, Tolypothrix* and *Aulosira* fix atmospheric nitrogen and are used as inoculants for paddy crop grown both under upland and low land conditions. *Anabaena* in association with water fern *Azolla* contributes nitrogen upto 60 kg/ha/season and also enriches soils with organic matter (Moore, 1969). In addition, a few cyanobacterial species form symbiotic associations with plants (algae, i.e., diatoms; fungi, i.e., lichens; bryophytes, i.e., liverworts, hornworts, and mosses; pteridophytes, i.e., *Azolla*; gymnosperms, i.e., cycads; and angiosperms, i.e., Gunnera), animals (marine sponges and achiruoid worms) non photosynthetic protists (belonging to the group Glaucophyta), bacteria, and hollow shafts of hairs of polar bears. The water fern *Azolla*, holding the N_2-fxing cyanobacterium, *Anabaena azollae*, is another established major cyanobacterial biofertilizer. Dry green algae contain high percentage of macronutrients, considerable amount of micronutrients and amino acids (El Fouly *et al*., 1992; Mahmoud, 2001). They can be conveniently produced on sewage and brackish water and partially substituted the chemical fertilizers to avoid environmental pollution. Kulk (1995) and Adam (1999) reported the growth promotion in response to application of nitrogen fixing cyanobacterium *Nostoc muscorum* could be attributed to the nitrogenase as well as nitrate reductase activities of cyanobacteria associated with the surface of plants, or the amino acids and peptides produced in cyanobacterial filtrate and/or other compounds that stimulated growth of crop plants. Besides being a source of N_2, BGA provides other advantages such as algal biomass accumulates as organic matter; produces growth promoting substances which stimulate growth of rice seedling; provides partial tolerance to pesticides and fungicides and also helps in reclamation of saline and alkaline soils.

Biofertilizer Production Technology

The success of any technology usually depends upon its techno economic feasibility. The algal production technology developed and reported by different algologists is very simple in operation and easy in adaptability by Indian farmers. The technology has got potential to provide an additional income from the sale of algal biofertilizer. The specific experimental steps of this technology is explained in fig.1. In general, there are four methods of algal production have been reported viz, (a) trough or tank method, (b) pit method, (c) field method and (d) nursery cum algal production method. The former two methods are essentially for individual farmers and latter two are for bulk production on a commercial scale.

a. *Trough or tank method*:

i. Preparation of shallow trays (2mx1mx23 cm) of galvanised iron sheet or permanent tank. The size of the tank can be increased if more material is to be produced.
ii. Spreading of 4 to 5kg of river soil and mixing well with 100g of superphosphate and 2g Sodium molybdate.
iii. 5 to 15cm of water poured in the trays. This will depend upon local conditions i.e. rate of evaporation. Then ingredients were mixed properly.
iv. In order to avoid the nuisance of mosquitoes and insects 10 to 15g Furadan granules or Malathion, or any other suitable granules was added.
v. The mixture of soil and water was allowed to settle for 8-10hours. At this time, 200 to 250g mother culture of blue green algae was added to the surface of water without disturbing the water.
vi. The reaction of the soil should be neutral. If the soil is acidic then $CaCO_3$ was added in order to bring the pH of the soil to neutral.
vii. If sunlight and temperature are normal then within 10-15 days the growth of the blue green algae will look hard flakes on the surface of the water/soil. Similarly, water level will be reduced due to evaporation.
viii. This way water in the tray/pit is allowed to evaporate and the growth of the algae flakes is allowed to dry
ix. If soil is dried the algal growth is separated from soil. These pieces of algal growth are collected and stored in plastic bags. In this way from one sq.m.tray or/pit about half tonnes kg blue green algal growth is obtained.
x. Again water was added to trays and stared the soil well. Then allow the algae to grow in this way. This time it is not necessary to add mother culture of algae or superphosphate. In this manner one can harvest growth of algae 2-3 times.

b. *Pit method:*

This method of production of blue green algae does not differ from the one described above i.e. trough method. Instead of troughs or tanks pits are dug in the ground and layered with thick polythene sheet to hold the water or one half cement plastered tanks. Other procedure is the same as in the trough method. This method is easy and less expensive to operate by small farmers.

c. *Field scale method:*

The field scale production of blue green algae is really a scaled up operation of trough method to produce the material on a commercial scale.

i. First the area in the field for algal production was demarcated. The suggested area is $40m^2$. No special preparation is necessary although algal production is envisaged immediately after crop harvest, the stubble is to be removed and if the soil is loamy it should be well puddle to facilitate water logging conditions.

ii. The area is covered with water to a depth of 2.5cm. In trough or pit methods flooding is done only in the beginning, while in field scale method flooding is repeatedly needed to keep the water standing.
iii. Then superphosphate 12kg/40m^{2}was applied.
iv. To control the insect-pests attack, carbofuran (3% granules) or Furadan 250g 40m^2 is applied.
v. If the field has received previously algal application for at least two consecutive cropping seasons no fresh algal application is required. Otherwise the composite algal culture of 5kg/40m^2.is applied.
vi. In clayey soils, good growth of algae takes place in about two weeks in clear, sunny weather, while in loamy soils it takes three to four weeks.
vii. Once the algae have grown and formed floating mats they are allowed to dry in the sun in the field and the dried algal flake, are then collected in sunny bags for further use.
viii. One can continually harvest algal growth from the same area by reflooding the plot and applying super phosphate and pesticides. In such situations an addition of algal inoculums for subsequent production is not necessary.
ix. During summer months (April-June), the average yield of algae per harvest ranges from 16-30kg/40m^2.

d. *Nursery cum algal production:*

Farmers can produce algae along with seedlings in their nurseries. If 320m^2 of land are allotted to prepare a nursery, an additional 40m^2 alongside can be prepared for algal production as described above. By the time rice seedlings are ready for transplantation about 15-20kg of algal material will be available. This much quantity of algal mass will be sufficient to inoculate one and half hectares of area. If every farmer produces the algal material required to inoculate his own land then he will reduce the cost of algal inoculums required to be purchased. So also one can cut the cost of chemical fertilizers to be applied as recommended.

Methods of Application of BGA Biofertilizer

One packet (500 g) of ready to use multani mitti based BGA biofertilizer is recommended for one acre of rice growing area. The packet is opened and mixed with 4 kg dried and sieved farm soil. The mixture is broadcast on standing water 3-6 days after transplantation. Use of excess algal material is not harmful; instead it accelerates the multiplication and establishment in the field. The field should be kept waterlogged for about 10-12 days after inoculation to allow good growth of BGA**.** When nitrogenous fertilizers are used, reduce the dose by one-third and supplement with BGA. Normal pest control measures and other management practices do not interfere with the establishment and activity of BGA in the field. Apply BGA for at least four consecutive seasons to have cumulative effect. One may not need to apply BGA further as these will establish in the field and reappear as and when the condition becomes favourable.

*Precautions***:** When fertilizer or pesticides (e.g. weedicides.) are applied in the field; the algal application should be followed after a gap of 3-4 days. Application of a small dose of phosphate fertilizer after BGA inoculation accelerates BGA multiplication. However, this quantity should be considered in the total application dose for rice corp.

Advantages of Using Biofertilizers

Biofertilizers are becoming a rage, considering the irreparable damage that the chemical fertilizers are causing to the soil. Some of the advantages associated with biofertilizers include:

- The first and the most important advantage of using biofertilizers is that they are environment friendly, unlike chemical fertilizers that damage the environment
- They are comparatively low on cost inputs and are light on the pockets of the farmers
- Their use leads to soil enrichment and the quality of the soil improves with time
- Though they do not show immediate results, but the results shown over time are extremely spectacular

- Microorganisms convert complex organic material into simple compounds, so that the plant can easily take up the nutrients
- These fertilizers harness atmospheric nitrogen and make it directly available to the plants
- They increase the phosphorous content of the soil by solubilising and releasing unavailable phosphorous
- Biofertilizers improve root proliferation due to the release of growth promoting hormones
- They help in increasing the crop yield by 10-25%

CONCLUSION

Biofertilizers have various benefits. Besides accessing nutrients, for current intake as well as residual, different biofertilizers also provide growth-promoting factors to plants and some have been successfully facilitating composting and effective recycling of solid wastes. By controlling soil borne diseases and improving the soil health and soil properties these organisms help not only in saving, but also in effectively utilising chemical fertilizers and result in higher yield rates. Cyanobacteria play a spectrum of remarkable roles in the field of biofertilizer, energy production, human food, animal feed, polysaccharides, biochemical, pharmaceutical and changing up of the environment, etc. The cyanobacteria provide inexpensive nitrogen to plants besides increasing crop yield by making the soil fertile and productive. BGA biofertilizer in rice popularly known as 'Algalization' helps in creating an environment friendly agro-ecosystem that ensures economic viability in paddy cultivation while saving energy intensive inputs. Cyanobacterial fertilizer also helps in the stabilization of soil, add organic matter, release growth promoting substances, improve the physico-chemical properties of soil and solubilize the insoluble phosphates. The technology can be easily adopted by farmers for multiplication at their own level.

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