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Plant growth promoting Cyanobacteria as potential biofertilizer and biocontrol agent in agriculture.

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1. ***Abstract**: With the emerging ascendancy of green revolution, irrational use of chemical herbicides, pesticides and fertilizers has increased soaring cases of cancer and detrimental effects on human and biodiversity worldwide. This urges search of alternatives. Possible cure all to an issue can be biofertilizer a powerful resource. This review presents cyanobacteria as promising renewable biofertilizer and bio control agent in today's agriculture. The article insights the role of cyanobacteria in soil to fix atmospheric nitrogen into utilizable form making it available to plants. The plant growth enhancement was observed due to hormones cytokinin, gibberellins and auxin. In addition, elicitor molecules like microcystin, antotoxin-a, cylindrospermopsin, cryptophycin, peptides, vitamins, carbohydrates are reported to induce pathogenesis. Aqueous and methanol cyanobacterial extract are conveyed to control various plant disease.*

Keywords: *BGA (Blue Green Algae), Cyanobacteria, bio control, biofertilizer, growth promotion*.

2. Introduction

With the skyscraping growth of population and proportional demand of food, green revolution no longer is capable to sustain the agricultural productivity. So to cope up with the increasing food demand it's high time to mobilize the available resources as rapidly and effectively as possible with minimal effect to environment. The panacea can be to bring more land under cultivation or enhance the productivity of cultivable land available by use of chemical fertilizers and biofertilizer [1, 2]. The first option of bringing more land into cultivation is not feasible because all the fertile lands are being converted into concrete buildings. Secondly the use of chemical fertilizers is discouraging. The unregulated profuse uses of chemical N and P fertilizer in remote areas and modern agriculture have invited several environmental hazards such as deterioration of soil quality, leaching, water pollution, reduced biodiversity , acidification, de nitrification and so on . That's why conscious and marginal farmers and consumers are shifting to renewable, economic and eco-friendly biofertilizer as an alternative to chemical fertilizer.

Biofertilizer are natural additives containing beneficial living microorganism. Nitrogen fixers, phosphorus solublizers like Pseudomonas, sulphur oxidizers, cellulolytic or organic matter decomposers like rhizobium, *Lactobacillus,* bacillus, A*zotobacter, Azospirillum* [3,4] , fungi of *Trichoderma*, yeast and blue green algae (BGA) are important constituents of biofertilizer. They colonizes the rhizosphere or the interior of the plant and promote plant growth and immunity by increasing the supply or availability of primary nutrient and/or growth stimulus to the target crop[5] . Multifarious micro-organisms present in biofertilizer [6] are presented in table 1 below.

Table 1: Micro-organisms present in biofertilizer

Table 1: Micro-organisms present in biofertilizer

SN	Micro-organisms	
	N2 fixer	*Rhizobium, Azotobacter, Azospirillu, Bradyrhizobium, Anabeana, Blue green algae such as Nostoc, Tolypothrix, Anabaena, and Aulosira, Cylindrospermum, Calothrix, Plectonema etc. Frankia* , N_2 fixing actinomycetes[6]
	Phosphorus solublizers	mycorrhizal fungi [7] Ecto rhizospheric *Pseudomonas and Bacilli*, and endosymbiotic rhizobia[8] *Pseudomonas, Bacillus, Rhizobium* and *Enterobacter a*long with *Penicillium* and *Aspergillus* fungi[9] fungus *Arthrobotrys oligospora* [10]
	Phosphorus mobilizer	Mycorrhiza (VAM vesicular arbuscular mycorrhiza= *Glomus fasciculatum*) [11]
	Plant growth promoter	*Pseudomonas, Trichoderma* [12]
	Fixed zinc solubilizer	*B.subtilis, Thiobacillus thioxidans* and *Saccharomyces sp*. [13]

Biofertilizer play important role in maintaining long term soil fertility and sustainability. It aids fixation of atmospheric dinitrogen (N=N) and aviability of insoluble phosphorus in the soil into forms available to plants, thereby increasing availability to plants[6].BGA/ Cyanobacteria are the best candidates to be selected as biofertilizer with ability to fix atmospheric dinitrogen. BGA enhances the diversity of beneficial normal soil micro flora resulting suppression of soil borne pathogens and disease providing tons of advantages over chemical fertilizer [1].

2.1. Blue green algae

Blue green algae (cyanobacteria) are gram negative photosynthetic, N_2 fixing prokaryote [14] capable of promoting plant growth rigorously has extensive history of being used as fertilizer. The presence of blue green pigment phycocyanin involved in photosynthesis entitles its name as cyanobacteria. They are found residing in all plausible habitat ranging from hot springs [15] , desert[16], hard rocks, fertile soil to fresh water and salt water in ocean[17,18,19]. A diversity of blue-green algae colonizes the detritus surface in the form of biofilms[20] Cyanobacteria thrive at temperature ranges 45-70^{0}C and pH 5-10[21,22]. Nitrogen fixing BGA like *Anabaena*, *Nostoc*, *Aulosira*, *Calothrix*, *Tolypothrix*, *Aphanothece* and *Gloeotrichia* are lavishly found in rice/paddy fields, but the distribution and abundance varies with soil and climatic condition. Blue green algae are capable of promoting plant growth with extensive history of being used as fertilizer as reported in following paragraph.

Nitrogen fixation, the microbial conversion of gaseous dinitrogen (N2) to ammonia is rendered by enzyme nitrogenase (E.C.1.18.6.1)[23] (Burris & Wilson, 1946). Heterocyst is specialized cell that cyanobacteria possess where nitrogen fixation occurs [24,25,26].It releases fixed nitrogen into soil either through exudation or through microbial decomposition after it's death[27] (Yagya Prasad Paudel & Pradhan, 2012). This ability of cyanobacteria makes them best candidate as biofertilizer. Dominant cyanobacterial nitrogen-fixer are *Anabaena, Nostoc, Aulosira, Cylindrospermum, Calothrix, Plectonema* etc [6].

Based on presence of heterocyst different types of cyanobacteria, various types of nitrogen fixing BGA are presented in table **2**. [28]

Table 2: Important nitrogen fixing cyanobacteria genera [1]

SN	Form of cyanobacteria	Cyanobacterial members
1	Unicellular	*Aphanothece, Chroococcidiopsis, Dermocapsa, Synechococcus, Gloeocapsa (Gloeothece)*∗*, Myxosarcina, Pleurocapsa*∗*, Xenococcus*
2	Filamentous heterocystous	*Anabaena*∗*, Anabaenopsis, Aulosira, Calothrix*∗*, Camptylonema, Chlorogloea, Chlorogloeopsis, Cylindrospermum, Fischerella*∗*, Gloeotrichia, Hapalosiphon, Mastigocladus, Nodularia, Nostoc*∗*, Nostochopsis, Rivularia, Scytonema*∗*, Scytonematopsis, Stigonema, Tolypothrix, Westiella, Westiellopsis*
3	Filamentous non-heterocystous	*Lyngbya, Microcoleus chthonoplastes, Myxosarcina, Oscillatoria, Plectonema boryanum, Pseudanabaena, Schizothrix, Trichodesmium*

Besides biological nitrogen fixation cyanobacteria also aids phosphate solubilization and mineral release to improve soil fertility [29]. A cyanobacterium helps to control stability, erosion, runoff via secretion of polysaccharides that firmly holds the soil [30]. BGA improves the physio-chemical parameters of the environment, in which they grow and flourish [31].

2.2. Cyanobacteria as bio control agent

Fungi, bacteria and viruses are biotic biocontrol agents that inhibits effects of the microbial pathogens in plant. The mode of action for bio control agents is antagonism, competition for nutrients and niches, prevention of colonization of host tissues by the pathogen and induction of resistance against the target diseases to be controlled[32].Cyanobacteria besides being a natural nitrogen fixer, they are paradigm of biocontrol agent combating multitude of plant pathogens via antagonism. They are equipped with potential to produce wide range of secondary metabolites [33] exhibiting antagonistic effects against different bacterial, viral and fungal plant pathogens [1]. Multifarious secondary metabolites with effective inhibitory action are potent fungicides, herbicides and insecticides [34,35]. Different strains and extracts of cyanobacteria in various solvent effective against disease causing plant pathogens is presented in table **3**.

Table 3: Cyanobacteria effective against various plants

SN	Cyanobacterial product	Causative agent of plant disease
1	Ether and water extract of *Nostoc muscorum*	*Sclerotinia sclerotiorum* infecting lettuce (*Lactuca sativa).*[36]
2	*Nostoc* Strain ATCC 53789	*Armillaria* sp., *Fusarium oxysporum* f. sp. *melonis*, *Penicillium expansum*, *Phytophthora cambivora*, *P. cinnamomi*, *Rhizoctonia solani*, *Rosellinia*, sp., *Sclerotinia sclerotiorum*, and *Verticillium albo-atrum*[37]
3	*Oscillatoria, Anabaena, Nostoc, Nodularia*	*Alternaria alternata and Botrytis cinerea*[38]
4	methanol extract of *Nostoc commune FK-103*	*Rhizopus stolonifer*
5	*Nostoc commune FK-103 and Oscillatoria tenuis FK-109*	*Phytophthora capsici*
6	*Anabaena species*	*Fusarium moniliforme* *Alternaria solani* *Drechslera oryzae* *Pythium aphanidermatum:* damping off, stem root rot [39]
7	Ethyl acetate fraction of *Azolla mycrophylla*	Against *Xanthomonas oryzae[40]*
8	Culture of N. muscorum.	checked an outbreak of damping off in millet-[41]
9	*Calothrix elenkenii*	*pythium aphanidermatum* damping-off disease[42]

The novel area for application of cyanobacteria is as insecticide and nematocide is tabulated below in table 4.

Table 1:Cyanobacteria as insecticide

SN	Cyanobacterial Bioactive substance	Function	Cyanobacteria species
	Microcystin	Algicidal/larvicidal/ herbicidal	*Microcystis aeruginosa, Calothrix sp.*[34]
	Anatoxin-a	Larvicidal	*Anabaena sp.*
	Cylindrospermopsin	Larvicidal	*Cylindrospermopsis sp.*
	Cryptophycin	*Armillaria* sp., *Penicillium expansum, Rosellinia* sp., and *Sclerotinia sclerotiorum*	Methanolic extracts of *Nostoc* strain ATCC 53789[37]
	Cyanobacterin (patented)	Phytotoxic and Herbicidal	*Scytonema hofmanni*, [43,44,45]

2.3. Cyanobacteria as growth promoter

Cyanobacteria are robust in nourishing and balancing nutrients and mineral in soil. It is competent enough to produce amino acids, vitamins, phytohormones acting as elicitor molecules to promote plant growth and induce immunity against biotic and abiotic stress.[1]. Plant growth promoting substances secreted by cyanobacteria is presented in table below.

Table 2: Plant growth promoting cyanobacteria

SN	Plant growth promoting substance	PGPR Cyanobacteria
1.	Cytokinin	*Calothrix,anabaena*[46,47]
2.	Giberellins	*Cylindromum, anabaenopsis Scytonema hofmanni*[48]
3.	amino acid histidine	Culture filtrates of cyanobacterial strains *C. ghosei, H. intricatus and Nostoc* [49]

2.4. Application of cyanobacteria in different crops

Many Asian countries like China, Vietnam, India, etc., have been utilizing cyanobacteria in paddy cultivation as the alternative to nitrogen fertilizers[50,51].Paddy field representing both terrestrial and aquatic ecosystem provides the best environment for cyanobacteria to fix N_2 profusely[52,53,54,55]. In favorable wetland conditions in paddy field, it is capable of accumulating 19-28 kg N/ha reducing the use of urea in rice culture by 25-35%[56,57].The mixed inoculum of BGA like *Nostoc, Anabaena, Westiellopsis, Aulosira* and *Scytonema* with N:P:K ratio (30:20:20 kg/ha) showed significant increase in rice productivity and nitrogen content in field[27,58].

The effect of cyanobacterial species of *Nostoc, Anabaena, Calothrix, Haplosiphon, Oscillatoria, Lyngbya,Phormidium*, isolated from the rhizosphere of diverse rice in non-flooded condition is indicated by increase in seed germination, root and shoot growth, weight and yield of the crop[29]. Enhancement of plant growth and yields in Chickpea(*Cicer arietinum* L.) through novel cyanobacterial and biofilmed inoculants is one of the recent significant output to prove the activity of cyanobacteria in non-flooded condition [59] (Bidyarani, Prasanna, Babu, Hossain, & Saxena, 2016).Growth promoting effect of cyanobacteria is now observed in wheat, cotton and several vegetables and herbaceous plants, including *Solanum lycopersicum, Cucurbita maxima, Cucumis sativus, Mentha spicata* and *Satureia hortensis*.[31.60]

Distinct endeavors were made to create artificial symbiosis between non leguminous plants and N_2 fixing micro-organisms [61]. Shoots were regenerated from tobacco callus cultures associated with the cyanobacterium A*nabaena variabilis* [62, 63]. Heterocystous cyanobacteria,*Nostoc* and *Anabaena* were associated with wheat seedlings in order to induce the ability of N2 fixation[64]. *Avicennia* seedlings colonized artificially with cyanobacteria productively promoted the growth of the seedlings[65].

2.5. Mass production of cyanobacteria

Watanabe and co-workers from Japan in 1971 ascertained the significance of BGA very firstly. Then, in India, numerous organizations and IARI carried out various researches on inoculation of BGA. Progressively, scientist at IARI New Delhi developed method for mass propagation of *Aulosira, Tolypothrix, Nostoc, Anabaena and Plectonema* [51]. AICPA (All India Co-ordinated Project on Algae) supplied cheaper dried starter consisting of *Aulosira, Anabaena, Nostoc, Plectonema and Tolypothrix* to farmers.

The application of BGA culture in field as biofertilizer /algalization was firstly introduced by Venkataraman in 1961. The algalization is practiced

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in Tamil Nadu and Uttar Pradesh, Jammu and Kashmir, Andhra Pradesh, Karnataka, Maharashtra and Haryana, China, Egypt, Philippines. Significant works are being done at Central Rice Research Institute, (Cuttack), Indian Council of Agricultural Research (New Delhi) and other centers, *e.g.* Centre of Advanced Study in Botany, Banaras Hindu University, Varanasi.

For the mass production of biofertilizer usually, effective strains of nitrogen-fixing, phosphate-solubilizing or cellulolytic microorganisms like *Azotobacter, Azospirillum, Rhizobium* are cultured in liquid medium in laboratory. These are used as inoculum for production in indoor or outdoor tanks and then the fresh biomass is loaded in an organic carrier materials like peat, soil, lignite, vermiculite, charcoal etc. [66,67]. The combination is packaged and stored followed by quality assessment.

The mass production of BGA is easily embraceable and economic for farmers. They can be conveniently produced on sewage and brackish water [6]. The reaction of the soil and water should be neutral. If the soil is acidic then addition of CaCO3 brings the pH of the soil to neutral. Profuse growth of algae takes place in clayey soil in about two weeks in clear, sunny weather, while in loamy soils it takes three to four weeks. During summer months (April-June), the average yield of algae per harvest ranges from 16-30kg/40m^2.

Four of the popular methods for propagation of BGA like *Tolypothrix, Aulosira, Anabaena, Nostoc and Plectonema [68]*(G. Venkataraman, 1981) are mentioned below. starting from laboratory to field are as follows: In laboratory, different nitrogen fixing BGA are maintained, cultured and multiplied in soil extract medium (1gm soil +10 ml Fogg's medium) which are later transferred to below mentioned method.

1. Trough or tank method
2. Pit method
3. Field method
4. Nursery cum algal production method.

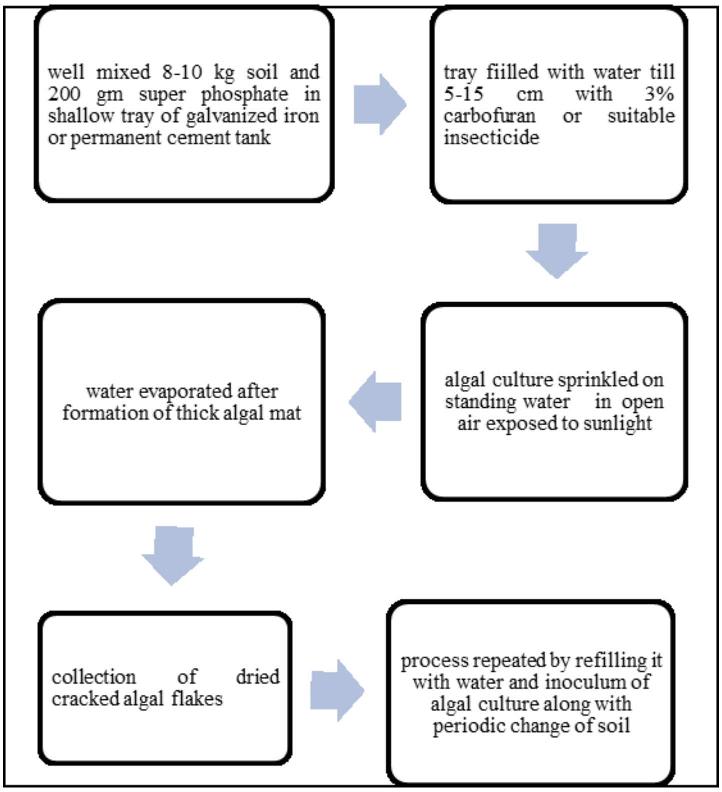

Figure 1.Viable commercial production of BGA [67]

1. Trough method

2. Pit method

The pit method is inexpensive and convenient for a farmer which is similar to the trough method. Here pit is dug, lined with polythene to hold water instead of tray or tank for propagation of blue green algae.

3. Field method

It is scaled up process of pit and trough method for commercial production of BGA.

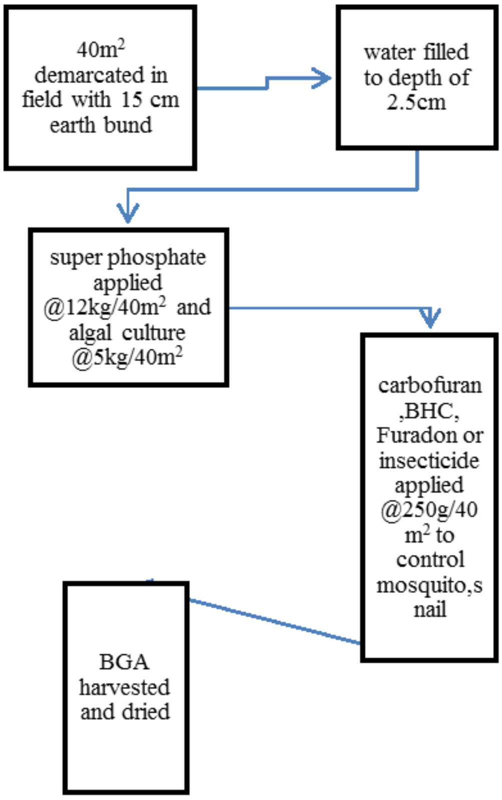

Figure 2:Pit and field method

4. Nursery cum method

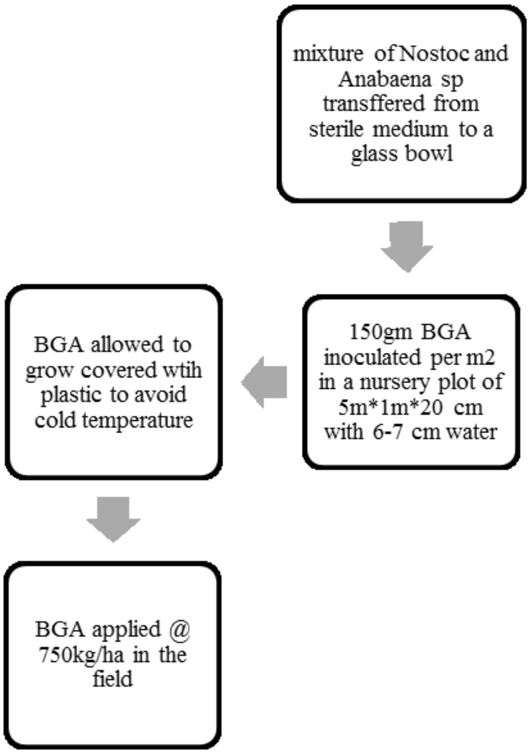

Figure 3:Nursery cum method

In this method farmers can produce BGA inoculum to spread in a rice field when they are growing seedling in their nurseries. By the time the rice seedlings are ready for transplantation, the sufficient inoculum of BGA is harvested by the above mentioned method.

2.6. Mass production of symbiotic cyanobacterium Anabaena holding Azolla

Azolla is a floating aquatic fern that occurs in ponds, ditches and paddy fields of warm-temperate and tropical regions throughout the world [69].The symbiotic relationship between the *Azolla* and cyanobacteria has been exploited for many years as a source of nitrogen for agriculture [70].The water fern *Azolla* found in tropical areas is potent major cyanobacterial biofertilizer harboring the N2-fxing cyanobacterium, *Anabaena azollae* [6]. *Anabaena* is invariably present in cavities in dorsal leaves contributing nitrogen up to 60 kg/ha/season [71]. BGA and *Azolla* brings about prominent and agronomic changes in the physical, chemical and biological properties of the soil and soil-water interface in rice field [31].
Azolla grows normally when it gets 25–50% full sunlight; slight shade is of benefit to Azolla growth in field condition. However, when the light intensity

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is lower than 1500 lux, the biomass production of Azolla will be greatly decreased [72]. Azolla can survive within a pH range of 3.5-10, but optimum growth is observed in the range of 4.5- 7.The most favorable temperature for growth and nitrogen fixation by *A. pinnata* is between 20-30ºC. Outside of this range, growth decreases until the plant begins to die at temperatures below 5ºC and above 45ºC.

Azolla nursery method for large scale multiplication of *Azolla microphylla* in the fields has been devised by Kannaiyan 1982 1989a.

1. The uniform thoroughly prepared plot of size 20*2m with suitable bunds and irrigation channel is prepared
2. Water is maintained at a depth of 10cm.
3. 10 kilos of fresh cattle dung is mixed in 20 liters of water
4. The mixture is poured into the plot and 8kg innoculum is inoculated into the plot
5. 100gm Super phosphate is applied in 3 split doses at the interval of 4 days as top dressing
6. Furadan granules at 100g/plot is applied 7 days after inoculation for insect pest control
7. harvesting is done 15 days after inoculation.

3. Conclusion

Effective utilization of ubiquitous cyanobacteria as biofertilizer will surely have indispensable effect on productivity of plants and soil. Substantial efforts and researches have been conducted to prove the effectiveness of BGA as biofertilizer aiding fertility to soil. However, like other chemical fertilizers, BGA is not a silver bullet to eliminate problems associated with depleted soils nutrient and hazards. However, keeping in mind it's properties useful in holistic management of soil fertility and disease in ecofriendly manner, use of BGA will surely pave the path towards sustainable agriculture. Several cyanobacterial secondary metabolites and allelochemicals have proven to be significant antimicrobial and are conspicuous target for commercial development as biocides (such as algaecides, herbicides and insecticides).

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