

Chapter

Formulations of BGA for Paddy Crop

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Abstract

Blue green algae (BGA) are prokaryotic phototrophic organisms that can fix the atmospheric nitrogen biologically, and were directly applied as a biofertilizers in agricultural fields specifically Paddy field. Since they are having the ability to fix nitrogen, they are formulated with various adsorbents for the purpose of enhancing the crop growth along with maintaining the soil fertility and other soil factors responsible for productivity. The present study revealed that the formulations of blue green algae isolated from paddy fields of southern districts with different adsorbents like alluvial soil, sand, charcoal, and powdered paddy straw. All the adsorbents mixed with blue green algae showed significant growth when compared to the control plant. This determined that the adsorbent formulated mixed blue green algae enhanced the paddy plant growth under greenhouse condition.

Keywords: BGA formulations, adsorbents, cyanobacteria, nitrogen fixers, natural biofertilizers

1. Introduction

Blue green algae are present abundantly in rice fields and are important in helping to maintain rice fields fertility through nitrogen fixation. They belong to a group of ubiquitous photosynthetic prokaryotes which possess the ability to synthesize Chlorophyll a and carry out an important role in nutrient recycling and the maintenance of organic matter in aquatic systems including lakes, rivers and wetland. Nitrogen fixing blue green algae are known to be a prominent component of the microbial population in wetland soils, especially rice fields, contributing significantly to the fertility as a natural bio-fertilizer.

Nitrogen fixation is one of the most important biological processes and, though, the atmosphere contains about 79% nitrogen, most of the plants cannot utilize it. They can utilize combined nitrogen, like ammonium, nitrate, nitrite; etc. This process is called biological nitrogen fixation.

Rice (*Oryza sativa*) is a monocot plant, of the grass family (Poaceae). As a cereal grain, it is the most popular cereal worldwide, serving as a staple food for 39 countries and nearly half of the world's population [1]. Globally rice is considered as a dietary energy source providing 22% of total energy intake [2]. Rice is the second highest worldwide produce and consumed staple food and increasing ratio of population demands more production of rice to meet its consumption [3].

Blue green algal species that thrive in rice fields release small quantities of ammonia as the major fertilizing product, and small nitrogenous polypeptides during active growth, whereas most of the fixed products are made available mainly



Figure 1.
Effect of different formulations of mixed blue green algae on paddy plants under greenhouse condition.

through autolysis and decomposition. They have an important role to play in crop production as promising biofertilizers. Here an attempt was made to study the different formulations of blue green algae from the paddy field with the following objectives: Isolation and mass culturing of blue green algae from the areas of selective southern districts of Tamil Nadu. The selective isolated blue green algae have been formulated with different adsorbent like alluvial soil, sand, charcoal, powdered paddy straw and analyzed the interaction effect of various for BGA on vegetative growth of paddy plant (**Figure 1**).

2. Materials and methods

2.1 Sampling

The soil samples collected from the areas namely as Thiruvadanai, of Ramnad, Selugai and Amaravathipuram of Sivagangai and Sakkimangalam of



Figure 2.
Sampling sites.

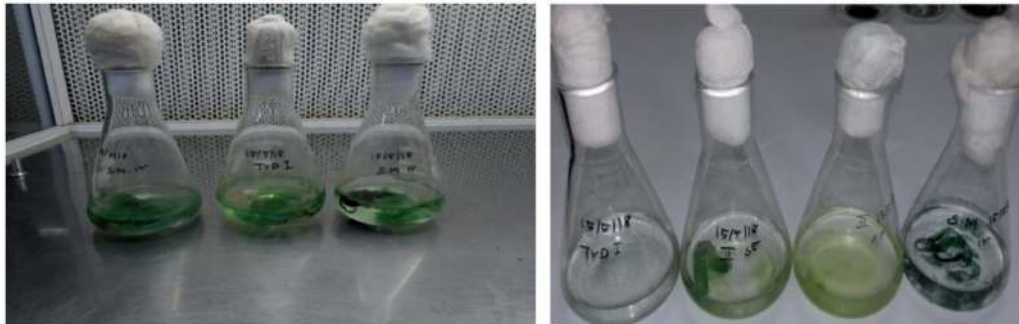


Figure 3.
Sub culturing of isolated blue green algae.

Madurai (Figure 2). They were stored at room temperature and were used as samples for further research.

2.2 Culture techniques

The BG₁₁ with nitrate and without nitrate medium was prepared and sterilized in autoclave for 121°C, 15 lb pressure for 20 min. After cooling, the samples were inoculated in the BG₁₁ medium for enrichment. The inoculated flasks were maintained at a temperature of 25°C and 12 h light and 12 h darkness (light intensity 3000 lux).

2.3 Identifying and sub culturing

The blue green algal growth was observed and identifying the organisms under Labomed vision 2000 smart scope B6. The selective identified organisms were sub cultured in BG₀ under lab and maintained for further analysis (Figure 3).

2.4 Formulations of BGA

The BGA mixture (10 ml of each *Microcoleus*, *Microcystis*, *Phormidium* and *Gloecapsa*) was added with 50 g of selective adsorbents (alluvial soil, sand, charcoal, powdered paddy straw). Then such combinations were shade dried under laboratory condition. After drying, such mixture was packed in polythene bags further study.

3. Paddy plant selected for general greenhouse procedure

Seeds of Paddy variety CR-1009 were surface sterilized with hot water for 5 min and washed with sterile water repeatedly. Then these seeds were placed in hot water for 10 min to soften the seed coat. Sterile garden soil was used to fill the earthen pots 15 cm height; 52 cm diameter. About 5 kg of sterile soil were taken in each earthen pot which was mixed with different adsorbent formulated BGA. Seeds (15 Nos.) were sown in each pot and germinated seedlings were thinned out to 10 in each pot. The above experimental plants were maintained under greenhouse conditions. The sterilized tap water was used for irrigating the plants. Such experimental pots were assigned for the following treatments:

- C—control (without organism)
- T1—alluvial soil + mixed BGA

- T2—sand + mixed BGA
- T3—charcoal + mixed BGA
- T4—powdered paddy straw + mixed BGA

3.1 Determination of growth

The paddy plant vegetative growth (15th day) was measured with the following growth parameters.

3.2 Determination of fresh and dry weight

The plant materials were cut into bits and weighed. Then they were dried in an oven at 90°C until the weight became constant.

3.3 Shoot and root length determination

The shoot and root lengths of the plants were measured using a meter-scale.

3.4 Determination of leaf number

The number of leaves or leaflets was counted for each plant.

3.5 Estimation of chlorophyll

The experimental leaf tissue was estimated for chlorophyll by following the method of Arnon [4]. Fifty milligram of Leaf tissue was homogenized in 80% pre chilled acetone by using a mortar and pestle and centrifuged at 3000 rpm. The pellet was homogenized again with acetone and was centrifuged repeatedly till the pellet become pale. The collected supernatants were pooled and the absorbance of the supernatant was read at 645 and 663 nm.

The chlorophyll content (mg/g fr.wt) was calculated by using the following formula:

$$\text{Total chlorophyll (mg/g fr.wt) content} = \frac{22.4 \times A_{645} + 8.02 \times A_{663}}{l \times 1000 \times W} \times V$$

$$\text{Chlorophyll-a (mg/g fr.wt) content} = \frac{22.9 \times A_{663} - 2.69 \times A_{645}}{l \times 1000 \times W} \times V$$

$$\text{Chlorophyll-b (mg/g fr.wt)} = \frac{22.9 \times A_{645} - 4.68 \times A_{663}}{l \times 1000 \times W} \times V$$

where l is the path of light length in cm (1 cm), V is the volume of the extract in ml and W is the fresh weight of the sample in g (Chlorophyll contents were expressed either as mg or μg for the plant samples).

3.6 Protein estimation

The experimental fresh leaf tissue of the protein content was estimated by Lowry's method [5]. About 50 mg of the leaf tissue was weighed and was homogenized in hot 80% ethanol and macerate in a mortar with pestle. The supernatant was discarded and the pellet was collected for the analysis purpose. The collected

pellet was suspended in a suitable volume of 5% TCA in an ice-bath for 15 min. The pellet was re extracted once in hot absolute ethanol and twice with ethanol-ether mixture, every time discarding the supernatants after centrifugation. Such collected pellet contained proteins and nucleic acids.

The extracted protein sample was placed in 1 ml of sodium hydroxide at 100°C for 4–5 min. The alkaline copper reagent (5 ml) was added and allowed to stand at room temperature for 10 min. Then the folin phenol reagent (0.5 ml) was added rapidly and mixed immediately. After 30 min, the absorbance was measured at 750 nm in a UV-Visible Spectrophotometer. The quantity of protein in the sample was calculated with a standard curve prepared using bovine serum albumin of different concentrations.

3.7 Statistical analysis

The data collected in this study was subjected to statistical methods standard deviation bar charts and pie charts applied [6].

4. Results and discussion

Blue green algae (cyanobacteria) play an important role in maintenance and build-up of soil fertility, consequently increasing rice growth and yield as a natural biofertilizer [7]. They are photosynthetic nitrogen fixers and are free living. Increase in water-holding capacity through their jelly structure [8].

Cyanobacteria are known to be one of the promising supplements to nitrogenous fertilizer, but the process biological nitrogen fixation, mediated through the enzyme nitrogenase may be inhibited in presence readily available nitrogen source. Supplementation of chemical fertilizer with blue green algae could conserve up to 30% of commercial fertilizer and it is generally believed that the nitrogen fixed by these organisms is made available to the rice plants through exudation or autolysis and microbial decomposition. Onkar et al. [9] in addition to contributing fixed nitrogen and adding organic matter to soil such blue green algae are also known to excrete growth promoting substances, solubilize insoluble phosphates, improve fertilizer use efficiency of crop plants and amend the physical and chemical properties of soils, increasing soil aggregate size, there by correcting soil compaction, reduce oxidizable matter of the soil and narrowing down the C:N ratio [10].

Nitrogen fixing filamentous cyanobacteria occurs in wide range of habitats mainly rice-field ecosystem and agricultural fields [11, 12]. In rice field among photosynthetic aquatic organisms, investigations have been emphasized more on isolation and identification of nitrogen fixing cyanobacterial populations in agro-ecosystems for sustainable agriculture.

Shelf-life of cyanobacteria biofertilizer can be augmented by selecting translucent packing material, dry mixing and paddy straw as a carrier [13]. Conventionally, soil has been used as a carrier for cyanobacterial biofertilizers whereas in one study it was reported that soil based inoculums have proved to be disadvantages due to poor inoculums loading, heavy contamination and its bulky nature [14–16]. Sugar cane waste; rice husk [17] and coconut coir [18] was developed as new carrier material [13]. Field trials conducted using straw based, soil based and multani mitti based BGA biofertilizer and it was reported that multani mitti based biofertilizer gave highest yield followed by straw based and soil based BGA inoculants [19].

In the present study the paddy field soil was collected from four different villages namely as Thiruvadana of Ramnad, Selugai and Amaravathipudur of Sivagangai and Sakkimangalam of Madurai district and blue green algae were

isolated as *Microcoleus*, *Microcystis*, *Phormidium* and *Gloecapsa* (**Figure 4**). These isolates were mixed and formulated in four different adsorbents—alluvial soil, sand, charcoal, and powdered paddy straw. The efficiency of such formulated blue green algae mixture on the morphological and physiological activity of paddy plant (15th day growth) was analyzed (**Table 1**). According to this all the formulated BGA (blue green algae) inoculated paddy plant showed progressive increase in shoot and root length, fresh and dry weight, number of leaves, chlorophyll and protein content when compared to control plant. Among these formulations the alluvial soil + BGA treated plants showed better growth by means of increase in chlorophyll and protein content which indicated that the photosynthetic and metabolic activity was enhanced due to this treatment. Blue green algae formulated with adsorbents influenced the paddy plant growth and also they contributed to improve the nitrogen fertility in soil.

The shoot and root length and fresh and dry weight of the paddy plant treated with alluvial soil + Mixed BGA and powdered paddy straw+ Mixed BGA showed maximum (18.83 ± 0.29 ; 3.93 ± 0.12 cm and 0.21 ± 0.00 ; 0.052 ± 0.00 g & 18.13 ± 0.12 ; 3.87 ± 0.12 cm and 0.209 ± 0.001 ; 0.051 ± 0.00 g) growth when compared to control (13.17 ± 0.29 ; 2.13 ± 0.23 cm and 0.17 ± 0.00 ; 0.043 ± 0.00). The number of leaves in all treated plants including control was more or less same (2 or 3). But the chlorophyll *a*, *b* and total chlorophyll content was higher in (0.485 ± 0.001 ; 0.1513 ± 0.001 ; 0.1803 ± 0.001 μg) alluvial soil + Mixed BGA and charcoal + mixed BGA (0.388 ± 0.001 ; 0.104 ± 0.001 ; 0.140 ± 0.000 μg) compared to control plant (0.0313 ± 0.001 ; 0.0187 ± 0.001 ; 0.0377 ± 0.001 μg) (**Table 1** and

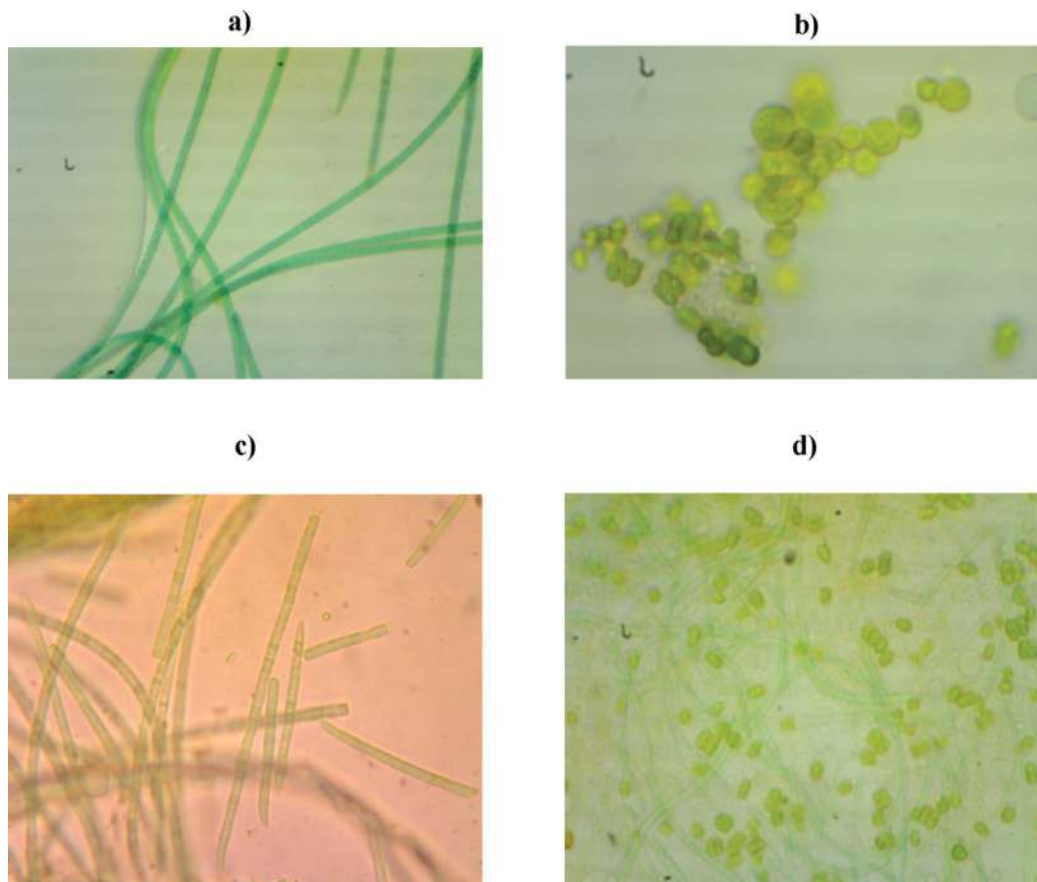


Figure 4. Microscopic view of isolated blue green algae from soil samples of sampling paddy fields. (a) *Microcoleus*, (b) *Microcystis*, (c) *Phormidium*, and (d) *Gloecapsa*.

Growth parameters	Control	T1	T2	T3	T4
Shoot length (cm)	13.17 ± 0.29	18.83 ± 0.29	15.5 ± 0.50	17.43 ± 0.40	18.13 ± 0.12
Root length (cm)	2.13 ± 0.23	3.93 ± 0.12	3.37 ± 0.12	3.6 ± 0.10	3.87 ± 0.12
No of leaves	2	3	2	3	3
Fresh weight (g)	0.17 ± 0.00	0.21 ± 0.00	0.20 ± 0.00	0.207 ± 0.00	0.209 ± 0.001
Dry weight (g)	0.043 ± 0.00	0.052 ± 0.00	0.0507 ± 0.00	0.043 ± 0.00	0.051 ± 0.00
Chlorophyll a (µg)	0.0313 ± 0.001	0.485 ± 0.001	0.251 ± 0.002	0.388 ± 0.001	0.279 ± 0.001
Chlorophyll b (µg)	0.0187 ± 0.001	0.1513 ± 0.001	0.074 ± 0.002	0.104 ± 0.001	0.080 ± 0.001

Values are mean of three replicates ± SD.

Table 1.
 Effect of different formulations of mixed blue green algae on the growth of Paddy plants under greenhouse condition.

Figure 5). The other formulated BGA treated plants showed minimal chlorophyll contents. The protein content of treated paddy plant with alluvial soil (28%; 2.52 ± 0.02 mg) + Mixed BGA and charcoal + mixed BGA (29%; 2.52 ± 0.00 mg) was significantly maximum when compare the control (10%) paddy plant (0.873 ± 0.06 mg) (**Figure 6**).

Katoh et al. [20] reported that *Nostoc* species are very useful in agricultural applications because of their nitrogen fixation activity, extracellular polysaccharide, photosynthetic system, and particularly desiccation tolerance ability and these properties help to improve the quality of nutrient poor soils. Wetland rice fields could provide an ideal condition for the growth of cyanobacteria, fixing $25\text{--}30$ kg N ha⁻¹ crop⁻¹, and reducing the use of urea fertilizer in rice culture by 30% [21, 22]. Algalization of BGA in rice cultivation promotes organic forming without usage of chemical fertilizers and production of organic basmati rice has been reported to develop a potential export market in the country [23].

Cyanobacteria also improve soil characteristics by modifying texture size and subsequent aeration and enhancing carbon content and water holding capacity [24]. Such organisms are one of the major components of the nitrogen fixing biomass in paddy fields. The importance of cyanobacteria in agriculture for paddy cultivation

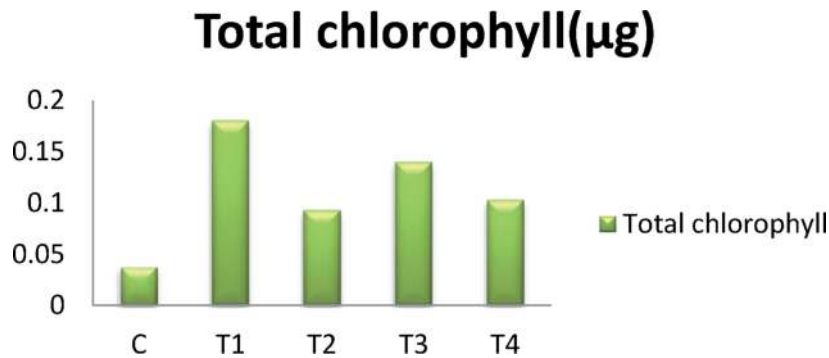


Figure 5. Effect of different formulations of mixed blue green algae on the total chlorophyll (μg) content of Paddy plants under greenhouse condition. C, control (without organism); T1, alluvial soil + mixed BGA; T2, sand + mixed BGA; T3, charcoal + mixed BGA; T4, powdered paddy straw + mixed BGA.

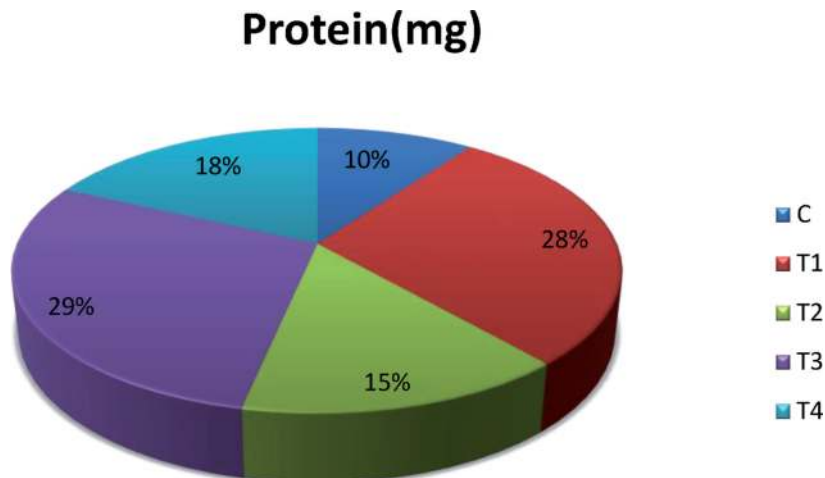


Figure 6. Effect of different formulations of mixed blue green algae on the protein content (mg) of Paddy plants under greenhouse condition. C, control (without organism); T1, alluvial soil + mixed BGA; T2, sand + mixed BGA; T3, charcoal + mixed BGA; T4, powdered paddy straw + mixed BGA.

is directly proportional to their ability to fix nitrogen and other positive effects for plants and soil. The nitrogen is the second limiting factor next to the water for plant growth in many fields and efficiency of this element is met by fertilizer [25].

Current study suggested that the efficiency of paddy plant growth was enhanced due to the application of formulated BGA with various adsorbents. Such blue green algae were generally applied as biofertilizers in agriculture for improving the soil fertility by the process of biological nitrogen fixation.

5. Conclusion

The blue green algae distributed in different environments. They are actively involved in the fixation of atmospheric nitrogen by the action of nitrogenase enzyme which is present in such organisms but not in plant cells. *Microcoleus*, *Microcystis*, *Phormidium* and *Gloecapsa*. were isolated from the paddy fields of Thiruvadanai, Selugai, Amaravathipudur, Sakkimangalam areas of Ramnad, Sivagangai and Madurai district. The isolated organisms were mass cultured under laboratory condition and mixed well. The BGA mixture formulated with alluvial soil, sand, charcoal and powdered paddy straw were treated on paddy plant showed significant growth compared to control plant. The present study concluded that the alluvial soil and powdered paddy straw formulated BGA promoted the plant growth by means of enhance the morphological growth but chlorophyll and protein content of the alluvial soil and charcoal formulated BGA treated plant showed was maximum. This indicated that the formulated BGA enhanced morphological and photosynthetic efficiency of the paddy plant under greenhouse condition. The application of such bio-mixture in agriculture for crop production not only increase crop yield which may maintain our environment eco-friendly.

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