

## Biofertilizers in minimizing climate change impacts in rice farming

**CLIMARICE:** " Sustaining the rice production in a changing climate uncertainties and validating selected adaptation techniques on farmer's field"

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This Technical brief is a short summary of the results obtained from the laboratory and field trials conducted by Climarice project team, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India to evaluate the potentials of microbial inoculants (biofertilizers) such as Blue Green Algae, Azolla and Phosphobacteria in minimizing the impacts of climate change in rice cultivation by nutrient supplementation, methane emission reduction and carbon sequestration. Cyanobacteria (Blue green algae) as a biofertilizer for rice in supplementing nitrogen is highly promising as the rice field ecosystem provides congenial environment for this self supporting diazotroph. Azolla is a floating water fern that also fixes atmospheric nitrogen. Azolla and Cyanobacteria have been identified as eco-friendly natural nitrogen fixers in the rice field ecosystem. A judicious use of these blue green algae could provide entire rice acreage of India as much nitrogen as obtained from 15-17 lakh tonnes of urea. Cyanobacterial nitrogen fixation helps to minimize the over dependence of chemicals, in particular, urea in rice farming and also enhances the use efficiency of nitrogen by releasing ammonia constantly to the rice crop.

In addition to nutrient supplementation, Cyanobacteria and Azolla that grow on the soil surface and also as a floating mass act as live aerators in paddy field ecosystem and oxygen released during the photosynthetic activity got liberated as minute air bubbles and consequently aerate the water impounded in paddy field that resulted in increased dissolved oxygen content which ultimately decreased the methane flux. In addition to the aeration of water, Cyanobacteria and Azolla might alleviate the toxicity due to accumulation of reduced iron and sulphites in rice fields observed under continued submergence. Recently, it has been reported that growing Cyanobacteria in rice fields results in sequestering carbon. Cyanobacteria are attractive candidates in this respect as they are fast growing and easier to manipulate in open ponds. They possess an essential biophysical mechanism (carbon concentrating mechanism, CCM), which concentrates CO<sub>2</sub> at the site of photosynthetic carboxylation. In this respect the

carbon concentrating mechanism (CCM) in cyanobacteria is similar to that found in C<sub>4</sub> land plants. This enables them to maintain high rates of CO<sub>2</sub> fixation, and also grow under low CO<sub>2</sub>. Hence, these Nitrogen fixing biological systems can be used to reduce methane flux from flooded rice ecosystem besides their ability in supplementing nitrogen to the paddy crop and sequestering carbon in rice soils.

### Biofertilizer in nutrient supplementation to rice crop

To study the role of biofertilizers such as Azospirillum, Blue Green algae and Azolla on nitrogen supplementation and rice yield a field experiment was carried out in the 'A1C' block of farm of Anbil Dharmalingam Agricultural College and Research Institute, Trichy during June-September, 2010. The farm is situated at 10°45'N latitude, 78°36'E longitude and at an altitude of 85 m above mean sea level. The experiment location having the climate of Semi-Arid Tropics experiences a mean annual rainfall of 843 mm distributed over 48 rainy days. The mean maximum temperature and minimum temperature are 34.8°C and 24.7°C respectively. The relative humidity ranged from 87 to 96 per cent in the forenoon and 66 to 87 per cent in the afternoon. The soil of the experimental field was sandy clay loam, taxonomically classified as isohyperthermic Vertic Ustropet, having 191 kg ha<sup>-1</sup> of available nitrogen, 27.5 kg ha<sup>-1</sup> of available phosphorus and 240 kg ha<sup>-1</sup> of available potassium. The rice variety TNAU (R) TRY1 was chosen for the study and it has duration of 135 days. The Green manure *Sesbania aculeata* was raised in a separate field and incorporated in the field as green leaf manure before planting as per the treatment.

**Fig. 1. Experimental applied with green leaf manure and farm yard manure**



Treatments involved viz., T<sub>1</sub>- Control, T<sub>2</sub>-Blue Green Algae, T<sub>3</sub>-Azolla, T<sub>4</sub>-Farm Yard Manure, T<sub>5</sub>-Green Leaf Manure, T<sub>6</sub>-Blue Green Algae+Azolla, T<sub>7</sub>-Farm Yard Manure + Green Leaf Manure, T<sub>8</sub>- Blue Green Algae + Azolla + Farm Yard Manure + Green Leaf Manure. A seed rate of 40 kg ha<sup>-1</sup> of rice variety (TRY1) was used for the experiment. The seeds were treated with Carbendazim @ 2 g kg<sup>-1</sup> of seeds for protection against seed borne diseases.

Fig. 2. Overview of experimental field



After 24 hours of fungicidal treatment, the seeds were treated with Azospirillum @ 600 g ha<sup>-1</sup> of seeds. The treated seeds were soaked in water for 24 hours to induce sprouting. The sprouted seeds were sown uniformly in the well prepared nursery maintaining thin film of water.

The results showed that rice yield was significantly higher in the plots applied with organic manure (FYM and GLM) and biofertilizers (BGA, Azospirillum and Azolla). Even though the methane flux is found to be high due to organics, application of organic manure is encouraged in rice cultivation due to higher yield and soil health.

In the present study, combined application of organics and biofertilizers not only recorded higher yield, but also found to emit less methane in paddy cultivation than the application of organics alone.

#### Methane flux reduction in rice soils by biofertilizers

The rice fields have to be considered as a significant source of greenhouse gases and rice field eco systems accounts for about 60 Tg methane per year or about 12 per cent of the global annual methane emission (IPCC, 1992). The emission of green house gases from rice ecosystem is likely to increase in the days to come due to intensification of rice cultivation.

Methane production and consumption in soil are the biological-mediated processes and therefore influenced by the prevalent weather condition, water regime, soil properties and various cultural practices like irrigation and drainage, organic amendments, fertilization and rice cultivars. Temperature, irrigation, redox potential, fertilization, available carbon and seasonal variations are among the factor that influences production of methane in soil (Allen *et al.*, 2003). Hence in the field experiment conducted at ADAC&RI, Trichy during June-September, 2012, the methane flux measurements were made to study the influence of biofertilizers such as BGA and Azolla on methane flux reduction. Soil temperature and water temperature were measured in each treatment during the entire crop period. Soil temperature reading was taken with mercury in glass thermometers (15 cm depth) which were placed in each treatment and water temperature was measured with ordinary thermometer. Soil and water temperatures were recorded at 10.00 and 15.00 hrs and averaged for the day.

#### Measurement of methane emission

Plant-mediated CH<sub>4</sub> emission flux from the experimental plots was measured by closed chamber method of Adhya *et al.* (1994) at regular intervals from transplanting to harvest. Samplings for CH<sub>4</sub> flux measurements were made at 09:00-10:00 hours and 15:00-16:00 hours and the average of morning and evening fluxes were used as the flux value for the day. For measuring CH<sub>4</sub> emission, eighteen rice hills were covered with a locally-fabricated transparent acrylic sheet chamber (59.3 cm length, 59.3 cm width and 87.8 cm height). A battery-operated fan was fixed for air circulation (avoid plant suffocation) to mix the air inside the chamber and draw the air samples into air-sampling bags (Tedlar®). The air samples from the sampling bags were analyzed for CH<sub>4</sub>. Each chamber was placed on the soil surface with 4-5 cm inserted into the soil, 10 minutes prior to each sampling for equilibration to reduce the disturbance to the sampling site.

Fig. 3. Experimental field with methane collection chamber

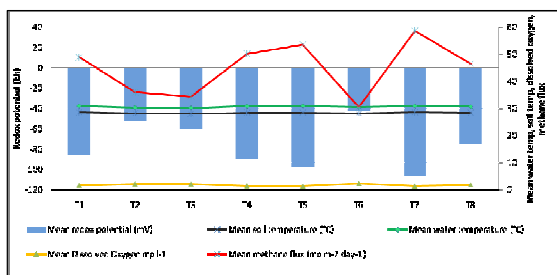


The CH<sub>4</sub> was estimated in a Shimadzu GC- 2014 gas chromatograph equipped with FID. The gas samples were introduced into the analyzer by filling the fixed loop (1.0 mL) on the sampling valve. Samples were injected into the column system by starting the analyzer which was automatically activates the valve and back flush the samples according to the time programmed. The retention time of CH<sub>4</sub> was between 4 to 4.17 min. The GC was calibrated before and after each set of measurements using 1 ppm, 2.3 ppm and 5 ppm of CH<sub>4</sub> (Chemtron® science laboratories Pvt. Ltd., Mumbai) as primary standard curve linear over the concentration ranges used. The minimum detectable limit for CH<sub>4</sub> was 1 ppm. CH<sub>4</sub> flux was determined by peak area and CH<sub>4</sub> flux was expressed as mg m<sup>-2</sup> day<sup>-1</sup> using the equation given by Lantin *et al.* (1995).

#### Influence of organic manure and photosynthetic systems on dissolved oxygen and methane flux

The BGA and Azolla application individually and in combination enhanced the dissolved oxygen concentration in the standing water in all growth stages while the dissolved oxygen concentration was minimum in farm yard manure and green leaf manure applied plots. BGA and Azolla are aerobic photosynthetic organisms and in the medium of their growth, they release a lot of oxygen during photosynthesis. As a result when they grow in rice fields they make the standing water highly oxygenated. When there is profuse growth of BGA and Azolla, the surface layer of the soil absorbs enough oxygen through diffusion to become aerobic in nature and prevents the development of highly reduced conditions underneath it. Mandal *et al.* (1998) and Lakshmanan *et al.* (1994) reported similar findings that BGA application increased the dissolved oxygen content in the standing water of rice field. Prasanna *et al.* (2002) also reported the beneficial effect of cyanobacteria in decreasing the headspace concentration of methane due to higher dissolved oxygen concentration that enhanced the methane oxidation at source.

Fig. 5. influence of temperature, redox potential, dissolved oxygen on methane flux



#### Effect of organic manure and photosynthetic systems on rice yield

Rice yield was significantly higher in the plots applied with organic manure (FYM and GLM) and biofertilizers (BGA and Azolla). Even though the methane flux is found to be high due to organics, application of organic manure is encouraged in rice cultivation due to higher yield and soil health. In the present study, combined application of organics and blue green algae not only recorded higher yield, but found to emit less methane in paddy cultivation than the application of organics alone. The mean methane flux in farm yard manure and green leaf manure applied plot was 58.54 mg m<sup>-2</sup> day<sup>-1</sup>, while the flux was reduced to 20 per cent due to BGA and Azolla application (46.37 mg m<sup>-2</sup> day<sup>-1</sup>).

Fig. 4. Mean methane flux and grain yield

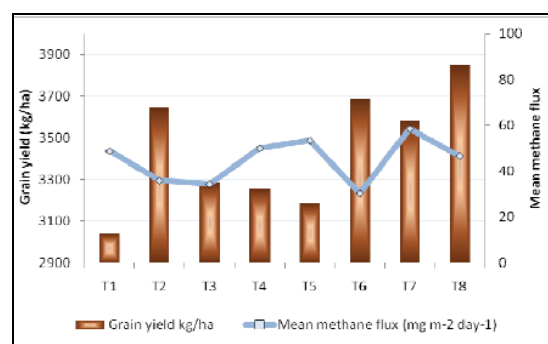


Table 1. Mean CH<sub>4</sub> flux, grain yield, straw yield and harvest index of experimental field

Tr. No	Mean CH <sub>4</sub> flux (mg m <sup>-2</sup> day <sup>-1</sup> )	Grain yield (kg ha <sup>-1</sup> )	Straw yield (kg ha <sup>-1</sup> )	Harvest Index
T <sub>1</sub>	48.81	3040	4668	39.4
T <sub>2</sub>	35.92	3646	5307	40.7
T <sub>3</sub>	34.35	3287	5172	38.9
T <sub>4</sub>	50.08	3255	5099	39.0
T <sub>5</sub>	53.49	3188	5013	38.9
T <sub>6</sub>	30.03	3685	5551	39.9
T <sub>7</sub>	58.54	3581	5250	40.5
T <sub>8</sub>	46.37	3847	5778	40
SEd		40.3	42.05	0.38
CD (P=0.05)		86.45	90.2	0.81

Bharati *et al.* (2000) emphasized that application



of BGA and Azolla reduced methane flux without reducing rice yields and can be used as a practical mitigation option for minimizing

the global warming potential of rice ecosystem.

#### Cyanobacteria in carbon sequestration

Various solutions have been proposed to mitigate the greenhouse effect of CO<sub>2</sub> emissions. One of the options currently being explored is the use of cyanobacteria as CO<sub>2</sub> sinks. Cyanobacteria have been identified as eco-friendly natural nitrogen fixers in the rice field ecosystem. The utilization of cyanobacteria (Blue green algae) as biofertilizer for rice in supplementing nitrogen is highly promising as the rice field ecosystem provides congenial environment for this self supporting diazotroph.

**Fig. 6. Blue green algal mat in paddy rhizosphere**



Recently, it has been reported that growing Cyanobacteria in rice fields results in sequestering carbon. Cyanobacteria are attractive candidates in this respect as they are fast growing and easier to manipulate in open ponds. They possess an essential biophysical mechanism (carbon concentrating mechanism, CCM), which concentrates CO<sub>2</sub> at the site of photosynthetic carboxylation. In this respect the carbon concentrating mechanism (CCM) in cyanobacteria is similar to that found in C<sub>4</sub> land plants. This enables them to maintain high rates of CO<sub>2</sub> fixation, and also grow under low CO<sub>2</sub> (Badger and Price, 2003). The pioneering studies of Kaplan *et al.* using *Anabaena variabilis*, demonstrated that cyanobacteria were capable of accumulating inorganic carbon, to such an extent that intracellular concentration could be 1000 folds more than the external environment. Since then DIC (Dissolved Inorganic Carbon) accumulation has been demonstrated in many cyanobacterial strains e.g. *Cocochloris peniocystis*, *Anacystis nidulans*, *Chlorogloeopsis sp.*, *Nostoc calcicola* etc. Cyanobacteria are

excellent model systems which can provide the biotechnologist with novel genes and biomolecules having diverse uses in agriculture, industry and environmental sustainability. These systems also hold promise as efficient contraptions for harnessing greenhouse gases such as CO<sub>2</sub> and methane.

#### Algal succession in Rice soil

Experiments were carried out at Agricultural College and Research institute, Trichy, TN, India during October- January, 2011 to study the algal succession in rice soil besides quantifying the biomass generation potential of different cyanobacterial species isolated from Cauvery basin. The cyanobacterial isolates were belonging to the genera *Nostoc*, *Anabaena*, *Westiellopsis* and *Plectonema* and composite inoculum of these cultures were inoculated @ 4 Kg per acre. The three main heterocystous genera (*Anabaena*, *Westiellopsis*, *Nostoc*) responded differently to levels of irradiation. Higher abundance of *Nostoc* coincided with lower abundance of the other two genera. *Anabaena* was the first cyanobacterial species to develop in field two weeks after transplantation of rice. Kannaiyan (1990) reported that nitrogen fixing forms namely *Scytonema*, *Aulosira*, *Nostoc*, *Aphanotheca*, *Oscillatoria*, *Plectonema* were dominant during tillering phase of rice crop. *Gleotrichia* and *Rivularia* were reported to occur during panicle initiation and flowering phase of rice. Many rice-field soils not only contain a high density of cyanobacteria, but possess visually obvious growths of cyanobacteria at (or floating above) the surface, during most part of the growth stages. Some of the common rice field cyanobacteria are *Anabaena*, *Aulosira*, *Calothrix*, *Gleotrichia*, *Cylindrospermum*, *Nostoc*, *Fischerella*, *Scytonema*, *Tolypothrix* and *Wolleea* (Rai *et al.*, 2000).

**Photo: (A. Lakshmana) Blue green algal blooms in a Clima Rice farmer field, Trichy**



In adopting a rational approach for the use and management of natural resources in sustainable agriculture, the biofertilizers hold vast potential. Biofertilizers are major components of sustainable farming and the biological nitrogen fixation (BNF) is a fascinating biological phenomenon studied extensively to provide low cost nitrogen and improve crop productivity. The utilization of cyanobacteria as a biofertilizer for rice is highly promising as the rice field ecosystem provides congenial environment for this self supporting diazotroph. Besides nitrogen fixation, these photosynthetic algal systems can also be employed for sequestering atmospheric carbon in paddy soils.

### Recommendations

Blue green algal systems and Azolla could minimize methane emission from flooded rice soils at the levels of production, transport and oxidation. These bio fertilizers as a dual crop minimize the global warming potential from flooded paddy apart from their ability to supply nitrogen to the paddy crop. Hence these biofertilizers can be applied to paddy crop as given below.

### Blue green algae:

**Doze:** 4 Kg of composite blue green algal inoculum per acre.

**Application procedures:** Mix 4 Kg of blue green algal inoculum in 50 Kg of vermicompost and apply around the root zone of the standing paddy crop during 10-15 days after transplanting.

**Photo: (A. Lakshmanan) Azolla nursery at farmer field, Tanjore**



### Azolla:

**Doze:** 100kg of wet azolla fronds per acre.

### Application procedures:

Broadcast 100 kg of fresh azolla fronds in the standing water during 10-15 days after transplanting.

### Cost of the technology

The multiplication of Blue green algae and Azolla needs small piece of land, minimum water facility and manpower. All these inputs are very much available in villages and no paid external inputs or building facilities are needed. The climatic conditions prevailing in the rice cultivating zones are conducive for quick Azolla and algal multiplication and ensure technical viability. Hence cultivation of these biofertilizers is truly a low cost technology and highly suited for easy adaptation by the rice farmers. The cost of production of one Kg of blue green algae would be INR 25.0 and one Kg of azolla is INR.3.0

### Technology dissemination

The mother inoculum of blue green algae and azolla are mass multiplied at the Research stations of Tamil Nadu Agricultural University. These mother cultures are supplied to the village self help groups and the volunteers of the self help groups will be given on campus training on the multiplication, processing and usage of these biofertilizers. In turn these self help groups have initiated the field level multiplication of these biofertilizers in their respective villages and supply them to the farmers at very affordable price. Periodical trainings and awareness camps are conducted at the villages by the ClimaRice Research Team to popularize the technology among the farming community.

### Photos (A.Lakshmanan) Technology dissemination among the farming community



## Conclusion

In the present study, combined application of organics and blue green algae not only recorded higher yield, but found to emit less methane in paddy cultivation than the application of organics alone. Application of BGA and *Azolla* reduced methane flux without reducing rice yields and can be used as a practical mitigation option for minimizing the global warming potential of rice ecosystem and enhancement by nitrogen fixation.

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## CLIMARICE Project (2010-2012)

*ClimaRice* is an integrated project that aims to assess the climate variability and its impacts on the water availability and rice production systems in the Cauvery and Krishna river basin of Tamil Nadu, India. The overall goal is to contribute to the regional and national adaption strategies to sustain rice production and ensure food security amidst changing climate. The partners are:

- Bioforsk - Norwegian Institute for Agricultural and Environmental Research (Project Co-ordinator)
- Tamil Nadu Agricultural University, Coimbatore, India
- International Pacific Research Institute, Hawaii, USA
- International Water Management Institute, IWMI, Hyderabad, India.

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