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Response of Organic Matrix Entrapped biofertilizers on Growth, Yield and soil properties of Rice (*Oryza sativa* L.)

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ABSTRACT— ***A consortium of biofertilizers (charcoal based commercial Azotobacter chroococcum and Bacillus subtilis) in free and organic matrix entrapped form was applied as sole nutrient in two different doses for cultivation of rice plant (Oryza sativa L.cv. Moti (IET-7328). To prepare organic matrix entrapped granular fertilizers, the biofertilizers were entrapped in organic matrix consisting of cow-dung, dried power of neem (Azadirachta indica) leaves and clay soil in 1:1:1 ratios and 15 % saresh (plant gum of Acacia sp.) which provided an artificial micro-environment to the biofertilizers microbes. An enhanced dose (two times) of biofertilizers increased the growth of rice plants as measured on 30, 60, 90, 120 DAT in terms of fresh and dry biomass of shoots and roots over the half dose of the same biofertilizers consortium. The entrapment of fertilizers in the organic matrix further increased the grain and straw yield over the non-entrapped form. The entrapped biofertilizers also enriched field soil by increasing its nutritional status over free form of biofertilizers. Results indicates that enhanced dose of biofertilizers can be developed as an effective alternative to the conventional chemical fertilizers for rice cultivation in semiarid subtropical agro-ecosystem by providing suitable carriers with eco-friendly and organic nutrient technologies.***

Keywords— *Azotobacter chroococcum, Bacillus subtilis,* entrapped biofertilizers, organic matrix

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1. INTRODUCTION

Rice (*Oryza sativa* L.) is an excellent source of carbohydrates and proteins and has been considered by FAO a strategic crop for food security of the world population due to its ample adaptation to climates and soils (FAO, 2006). It is well known the need for increasing global rice production to attend the food demand mainly of highly populated country like India. It has been estimated that the global rice production must reach the equivalent to 430 Million tons by the year 2030 (Timmer *et al*., 2010) and about 455 million of tons by the year 2050 (Mohanty *et al*., 2010). It is also expected an increase in the amount of chemical fertilizers to be applied (Gregory *et al*., 2010); including nitrogen (N) that is the most limiting nutrient for the rice crop. A specific problem with nitrogen management in lowland rice ecosystems is the poor nitrogen use efficiencies (NUE). Poor NUE for nitrogenous fertilizers results the loss of nutrients through various mechanisms such as volatilization, leaching and denitrification (Xiang *et al*., 2008, ZouHong-tao *et al*., 2009, Kiran *et al*., 2010, Soares *et al*., 2012).
Therefore it is important to find alternatives to reduce the use of N fertilizers applied to rice crop without decreasing the productivity and causing risks of environmental pollution. A number of alternatives have been implemented during the last decades, e.g. biofertilizers, integrated plant nutrient system (IPNS), and farm yard manure (FYM), slow / controlled release fertilizers (Peng *et al*. 2002, Singh *et al*. 2008, 2010, Cong *et al*., 2011, Grant *et al*. 2012).

The utilisation of biological nitrogen fixation (BNF) technology can decrease the use of urea-N, prevent the depletion of soil organic matter and reduce environmental pollution to a considerable extent (Jeyabal and Kuppuswamy 2001; Choudhury and Kennedy 2004; Kennedy et al. 2004).
Strains of *Azotobacter*, *Rhizobium*, *Bradyrhizobium*, *Azospirillum*, *Pseudomonas*, *Bacillus* and *Acetobacter* have been developed as biofertilizers. These bacteria exert beneficial effects on plant growth and development. It has been demonstrated that certain rice varieties respond positively to inoculation when selected diazotrophic strains from these species were used as biofertilizer (Tran Van et al., 2000; Govindarajan et al., 2008; Rodrigues et al., 2008; Ferreira *et al*., 2010). Slow and controlled release fertilizers are also produced by the technical interventions which reduce the nutrient losses and provide nutrients to the plants for a comparatively longer duration. It plays an important role in improving fertilizers use efficiency by plants, thereby mitigating environmental pollution and sustainable agriculture (*Zhao* et al., 2010). Though, Biofertilizers offers an economically attractive and ecologically sound alternative to the chemical

fertilizers for realizing the ultimate goal of increased productivity, its efficacy is significantly low in relation to the crop yield when compared with the recommended dose of chemical fertilizers.
Here, we have developed a low-cost, high efficient, sustainable organic matrix entrapped slow release biofertilizers, using local biodegradable agro waste like cow dung, clay soil, neem leaves easily available for Local production by small-scale industries or by farmers. The objective of this paper is to investigate the response of organic matrix entrapped biofertilizers on growth, yield and soil properties of rice plant.

2. MATERIAL AND METHODS

Experimental Design

The experiments were conducted in the environmental field station at Babasaheb Bhimrao Ambedkar University, Lucknow, India. Lucknow is situated at 123 m above sea level on 26.30^0 and 27.10^0 North latitude and 80.30^0 and 81.13^0 East longitude. The certified seeds of rice plant (*Oryza sativa* L.cv. Moti (IET-7328) was obtained from a local dealer of Lucknow. The experimental design was a randomized block of seven treatments replicated three times. The plot size was 1.5 m X 1 m. The treatments were 1) NF = no fertilizer , 2) FOM = free form of organic matrix, 3) EOM = entrapped form of organic matrix, 4) BF-I = Free form of recommended dose of (0.6 kg/ha) biofertilizers (*Azotobacter chroococcum* and *Bacillus subtilis* mixed with charcoal as carrier) in single dose, 5) OMEBF-I= Organic matrix entrapped biofertilizers in single dose, 6) BF-II= Free form of recommended dose of the biofertilizers in double dose , 7) OMEBF-II=Organic matrix entrapped biofertilizers in double dose

Entrapment of biofertilizers in organic matrix

Agro- waste like cow dung, neem (*Azadirachta indica*) leaves and clay soil (diameter of particles <0.002 mm) were collected locally. All the collected materials were dried separately in an oven at 60-70^0 C for 3 days and powdered in a grinder and mixer. The biofertilizers like *Azotobacter chroococcum* and *Bacillus subtilis* immobilized in charcoal as carrier were obtained for Biotech Park, Lucknow. These supporting matrixes were mixed in 1:1:1 ratio. Different doses of biofertilizers (*i.e.* 0.6 and 1.2 kg/ha) containing a consortium of nitrogen solubilizing bacteria (*e.g., Azotobacter*) and phosphate solubilizing bacteria (*e.g., Bacillus*) were mixed with the above organic materials and 15% commercial saresh (plant gum of *Acacia*), and small granules of approximately 5 mm diameter were prepared manually and dried at room temperature.

Soil and plant sampling and analysis

Both soil and plant samples were taken at 30, 60, 90 and 120 days after transplanting (DAT). Different plant growth parameters were measured. The plant parts removed carefully from the growing plants, washed with de-ionized water and dried by blotting it on filter paper. The fresh weight of roots and shoot were determined using single pan electrical balance. The tissues were oven dried at 70°C, till constant dry weight was recorded. Soil samples were collected from 0-20 cm. Soil layer, dried by venting, sieved and stored in loosely tied plastic bags to ensure sufficient aeration and prevent moisture loss prior to assaying different soil parameters. Physico-chemical properties of the soil were measured by the standard methods of soil chemical analysis followed by APHA (1984).
The soil dehydrogenase activity was measured by the method of Casida *et al*. (1964) and alkaline phosphatase activity in soil was measured by the method of Tabatabai and Bremner (1969). Microbial biomass was measured by plate count method described by American Public Health Association (1984).

Statistical Analysis

Analysis of variance (ANOVA) was employed followed by Duncan's Multi Range Test (DMRT) significant at $p<0.05$, to calculate the significance difference between control and experimental means. The results of the multi range test are presented in the figures and tables as the mean ± SD (n=6).

3. RESULTS AND DISCUSSION

Application of double dose of the biofertilizers (consortium of *Azotobacter chroococcum* and *Bacillus subtilis*) increased fresh weight and dry weight of shoots and roots significantly over no fertilizers application as well as the recommended dose of biofertilizers (Table 1).1.2 kg/ha biofertilizers in entrapped from caused a very significant increase in the plant biomass over the recommended dose (0.6 kg/ha) and non- entrapped biofertilizers. The increase in plant growth due to the entrapped and enhanced dose of biofertilizers was consistent at all four ages of the plants (30, 60, 90, 120 DAT).

Both the grain yield and straw yield of rice was influenced by different application methods of fertilizers. Application of OMEBFs increased grain and straw yield by more than four fold over no fertilizers. The application of BFs also increased the grain and straw yield but it was lower than OMEBFs. Grain yield was increased by about 3.19 and 4.29 fold with OMEBF-I and OMEBF-II followed by 2.85 and 3.59 fold with BF-I and BF-II over NF-applied plots. Straw yield was also increased by about 2.52 and 3.39 fold with OMEBF-I and OMEBF-II followed by 2.29 and 2.70 fold with BF-I and BF-II over NF-applied plots. In this study the highest grain yield (3.74 t ha^{-1}) and straw yield (4.04 t ha^{-1}) was observed from OMEBF-II (table 2). The free form of organic matrix (FOM) and its entrapped form (EOM) significantly affects the growth of rice over NF but less effective than BFs and OMEBFs.

Soil physico-chemical properties were significantly influenced with the application of fertilizers (Table 3). Application of OMEBF increased % OC, total and available N, available P and K over BF and NF. Single basal application of OMEBF-II showed increase in total N, available N, available P and soluble K over other treatments. It seems that the OMEBF fertilizer enhances the availability of nutrients in soil due to the little loss of nutrients from soil and slowly releasing process. Organic matrix also serves as a good nutrient source in soil. In rice soil, the application of slow release OMEBF significantly increased the soil microbes' population compared with BF and NF. Application of OMEBF significantly affected soil biological properties; fungal count, bacterial count, dehydrogenase activity and alkaline phosphatase activity over free biofertilizers and no fertilizer.

Dehydrogenase activity is commonly used as an indicator for biological activity, i.e., it can be used to indicate the total microbial population. On the other hand, the alkaline phosphatase activity for rice cultivated soil was being increased in all the treatments as compared to control plant. Results indicated that the application of agrochemicals significantly inhibits the population of phosphate solubilizers and nitrogen fixers, which was reported earlier by Balamurugan et al. (2010). It may be stated that the increase in phosphatase activity in OMEBFs treated soil is an indication of the increased soil fertility and improvement in the phosphate solubilization.

It appears that OMEBF is increasing efficacy of the commercial biofertilizers which is possible by increased in microbial population in the soil and increased availability of nutrients as evident from a similar increase in other growth and nutritional parameters. The data presented in this paper indicates that the application of biofertilizers consortium in double dose than the recommended dose and its entrapment in an organic matrix earlier used by us to entrap chemical fertilizers like urea and ammonium sulphate, increase the growth and yield of rice plant. (Dahiya et al., 2004, Sharma and Singh, 2011, Kumar, 2012)

The results shows that the recommended dose of biofertilizers for rice in semi-arid, subtropical Indo- Gangetic plain region of north Indian state is not a true reflection of the actual requirements of biofertilizers of different crops in different agro-climatic regions and it need a reconsider. In our case the double dose of biofertilizers provide better nutrients availability and crop productivity. In this case we have not optimized the optimal dose of this biofertilizers which is planned for future. In addition, entrapment of these biofertilizers to a biodegradable, low cost organic matrix contained local and cheap agro-waste materials like cow-dung, neem leaves powder, and clay soil and Acacia gum enhance its efficacy over the free form of biofertilizers. This opens a new aspect to improve and develop commercial biofertilizers which can sustain the crop productivity parallel to conventional chemical fertilizers and simultaneously can be eco-friendly, cost effective and soil enriching.

4. ACKNOWLEDGEMENT

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Appendix:

Table: 1- Effect of different fertilizer applications on the fresh and dry biomass of shoots and roots of rice plant on 30, 60, 90 and 120 DAT

	Treatments	30d	60d	90d	120d
Fresh weight of shoot (g)	NF	2.78±0.03^{d}	5.05±0.23^{e}	12.67±1.15^{f}	48.33±2.89^{e}
	FOM	3.90±0.04^{bc}	6.33±0.63^{d}	16.17±0.89^{e}	81.67±2.89^{d}
	EOM	3.88±0.07^{c}	7.40±0.46^{c}	18.30±0.36^{d}	86.67±2.89^{d}
	BF-I	3.93±0.06^{bc}	7.51±1.17^{c}	18.91±0.12^{d}	88.33±2.89^{d}
	OMEBF-I	3.81±0.09^{c}	9.30±0.56^{b}	20.92±0.94^{c}	101.67±7.64^{c}
	BF-II	4.35±0.15^{a}	9.08±0.23^{b}	26.83±1.03^{b}	111.67±2.89^{b}
	OMEBF-II	4.07±0.14^{b}	10.41±0.34^{a}	33.43±1.40^{a}	125.00±5.00^{a}
Dry weight of shoot (g)	NF	0.64 ±0.07^{d}	0.90±0.03^{c}	2.34±0.11^{e}	8.38±0.06^{g}
	FOM	0.90 ±0.02^{abc}	1.34±0.01^{b}	3.60±0.16^{d}	9.89±0.02^{f}
	EOM	0.86 ±0.03^{bc}	1.38±0.04^{b}	4.44±0.04^{c}	10.59±0.22^{e}
	BF-I	0.91 ±0.02^{abc}	1.40±0.02^{b}	4.56±0.23^{bc}	10.99±0.23^{d}
	OMEBF-I	0.84 ±0.01^{c}	1.57±0.03^{b}	4.75±0.05^{ab}	11.60±0.31^{c}
	BF-II	0.96 ±0.02^{a}	1.67±0.05^{ab}	4.85±0.06^{a}	11.91±0.07^{b}
	OMEBF-II	0.93 ±0.05^{ab}	1.95±0.07^{a}	4.89±0.01^{a}	13.89±0.11^{a}
Fresh weight of roots (g)	NF	0.64±0.04^{c}	0.92±0.02^{d}	2.34±0.11^{e}	8.38±0.06^{f}
	FOM	0.90±0.05^{b}	1.36±0.02^{c}	3.58±0.16^{d}	9.82±0.12^{e}
	EOM	0.88±0.03^{b}	1.40±0.03^{c}	4.44±0.21^{c}	10.42±0.22^{d}
	BF-I	0.92±0.04^{ab}	1.40±0.02^{c}	4.56±0.23^{bc}	10.92±0.23^{c}
	OMEBF-I	0.90±0.03^{ab}	1.57±0.03^{b}	4.75±0.15^{ab}	11.60±0.31^{b}
	BF-II	0.96±0.04^{a}	1.67±0.03^{b}	4.85±0.16^{a}	11.91±0.07^{ab}
	OMEBF-II	0.90±0.03^{b}	1.94±0.15^{a}	4.89±0.21^{a}	11.98±0.03^{a}
Dry weight of roots (g)	NF	0.04±0.01^{c}	0.11±0.02^{d}	0.32±0.04^{d}	0.74±0.06^{d}
	FOM	0.06±0.02^{b}	0.21±0.02^{c}	0.45±0.03^{c}	0.85±0.04^{c}
	EOM	0.07±0.02^{b}	0.26±0.04^{b}	0.51±0.04^{b}	0.90±0.03^{bc}
	BF-I	0.07±0.02^{b}	0.28±0.02^{b}	0.53±0.02^{b}	0.92±0.02^{b}
	OMEBF-I	0.07±0.02^{b}	0.26±0.02^{b}	0.54±0.02^{b}	0.95±0.03^{ab}
	BF-II	0.10±0.01^{a}	0.30±0.02^{b}	0.56±0.04^{b}	0.95±0.02^{ab}
	OMEBF-II	0.10±0.02^{a}	0.36±0.02^{a}	0.63±0.02^{a}	0.98±0.02^{a}

Values are means ±SD, (one way ANOVA) DMRT significant at $p<0.05$, followed by the same letter(s) are not significantly different at $p<0.05$

Where, NF = no fertilizer, FOM = free form of organic matrix, EOM = entrapped form of organic matrix, BF-I = Free form of recommended dose of (0.6 kg/ha) biofertilizers (*Azotobacter chroococcum* and *Bacillus subtilis* mixed with charcoal as carrier) in single dose, OMEBF-I= Organic matrix entrapped biofertilizers in single dose, BF-II= Free form of recommended dose of the biofertilizers in double dose, OMEBF-II=Organic matrix entrapped biofertilizers in double dose

Table 2- Effect of different fertilizer applications on grain and straw yield (t ha^{-1}) of rice plant

	yield		
Treatments	Grain yield (t ha^{-1})	Straw yield (t ha^{-1})	Harvest index (%)
NF	0.87±0.02^{g}	1.19±0.06^{f}	42.23
OM	1.88± 0.04^{f}	2.00±0.09^{e}	48.45
EOM	2.13±0.13^{e}	2.48±0.20^{d}	46.20
BF-I	2.48±0.12^{d}	2.73±0.21^{cd}	47.60
OMEBF-I	2.78±0.07^{c}	3.00±0.25^{bc}	48.09
BF-II	3.13±0.13^{b}	3.22±0.23^{b}	49.29
OMEBF-II	3.74±0.25^{a}	4.04±0.09^{a}	48.07

Values are means ±SD, (one way ANOVA) DMRT significant at $p<0.05$, followed by the same letter(s) are not significantly different at $p<0.05$

Where, NF = no fertilizer, FOM = free form of organic matrix, EOM = entrapped form of organic matrix, BF-I = Free form of recommended dose of (0.6 kg/ha) biofertilizers (*Azotobacter chroococcum* and *Bacillus subtilis* mixed with charcoal as carrier) in single dose, OMEBF-I= Organic matrix entrapped biofertilizers in single dose, BF-II= Free form of recommended dose of the biofertilizers in double dose, OMEBF-II=Organic matrix entrapped biofertilizers in double dose

Table 3. Effect of different fertilizer application on microbial properties and enzyme activity of soil in experimental field of rice crop before transplanting and after harvesting

	log no. of fungal colonies per g. soil	log no. of bacterial colonies per g. soil	Soil dehydrogenase activity(µg TPF g^{-1} soil per h^{-1})	Alkaline phosphatase activity (µg PNPP g^{-1} soil per h^{-1})
Experimental field before transplanting of rice crop	0.65	1.6	2.4	7.6
Experimental field after harvesting of rice crop				
NF	1.3 ^{d} ± 0.57	2.3^{d} ± 1.00	2.95^{f} ± 0.48	8.29^{e}±1.36
FOM	1.6^{C} ± 0.57	2.6^{d} ± 0.57	4.14^{e} ± 0.37	13.15^{d}±1.32
EOM	1.6 ^{c} ± 1.15	3.3^{c} ± 0.57	4.99^{e} ± 0.16	12.75^{d}±2.98
BF-I	2.0^{b} ± 0.00	3.6^{c} ± 1.00	8.51^{d} ± 1.00	16.74^{c}±0.95
OMEBF-I	2.3 ^{a} ± 1.15	4.0^{a} ± 0.57	15.08^{c} ± 3.37	18.12^{b}±0.21
BF-II	2.6^{b} ± 0.57	4.3^{b} ± 0.57	14.97 ^{b}± 0.62	20.02^{ab}±0.86
OMEBF-II	3.0 ^{a} ± 1.00	5.6^{a} ± 0.57	16.62^{a} ± 0.29	22.05^{a}±0.64

Values are means ±SD, (one way ANOVA) DMRT significant at $p<0.05$, followed by the same letter(s) are not significantly different at $p<0.05$

Where, NF = no fertilizer, FOM = free form of organic matrix, EOM = entrapped form of organic matrix, BF-I = Free form of recommended dose of (0.6 kg/ha) biofertilizers (*Azotobacter chroococcum* and *Bacillus subtilis* mixed with charcoal as carrier) in single dose, OMEBF-I= Organic matrix entrapped biofertilizers in single dose, BF-II= Free form of recommended dose of the biofertilizers in double dose, OMEBF-II=Organic matrix entrapped biofertilizers in double dose

Table 4. Effect of different fertilizer application on physicochemical characteristics of soil in experimental field of rice crop before transplanting and after harvesting

Treatments	pH	EC(Ds m^{-1})	OC (%)	Total N(kg ha^{-1})	Available N (kg ha^{-1})	Available P (kg ha^{-1})	Available K (kg ha^{-1})
Before Transplanting of rice crop							
	8.55 ±0.06.	0.26±0.01	0.35±0.04	933.67 ±14.19	151.67± 10.41	12.73 ± 1.67	212.51 ±7.40
After harvesting of rice crop							
NF	7.75 ± 0.05	0.20±0.03	0.37 ±0.03	940.72 ±11.10	156.67 ± 4.16	13.84 ± 0.85	218.81 ± 5.11
FOM	7.55 ± 0.15	0.22±0.02	0.41 ± 0.05	951.00 ±14.11	170.00±5.29	16.07 ± 1.61	223.40±15.60
EOM	7.27±0.04	0.24±0.01	0.47 ±0.02	955.00±10.15	213.67±7.64	19.76 ±0.73	230.18±10.23
BF-I	7.43 ± 0.05	0.23±0.06	0.57 ± 0.06	961.33±17.99	229.00±5.00	26.49 ±1.95	251.03±12.38
OMEBF-I	8.08 ±0.13	0.24±0.03	0.64± 0.05	965.00±15.78	247.33±7.64	29.19 ± 1.34	257.43±15.84
BF-II	7.86± 0.06	0.25±0.01	0.67± 0.06	975.0 ±15.01	255.33±8.32	30.82 ±2.36	278.35 ±17.25
OMEBF-II	7.95 ±0.10	0.25±0.01	0.71± 0.06	980.0±10.02	260.00±8.77	35.62 ±2.19	286.60 ±17.76

Where, NF = no fertilizer, FOM = free form of organic matrix, EOM = entrapped form of organic matrix, BF-I = Free form of recommended dose of (0.6 kg/ha) biofertilizers (*Azotobacter chroococcum* and *Bacillus subtilis* mixed with charcoal as carrier) in single dose, OMEBF-I= Organic matrix entrapped biofertilizers in single dose, BF-II= Free form of recommended dose of the biofertilizers in double dose, OMEBF-II=Organic matrix entrapped biofertilizers in double dose

Figure1: organic matrix entrapped slow release fertilizers

Figure 2: Rice plant

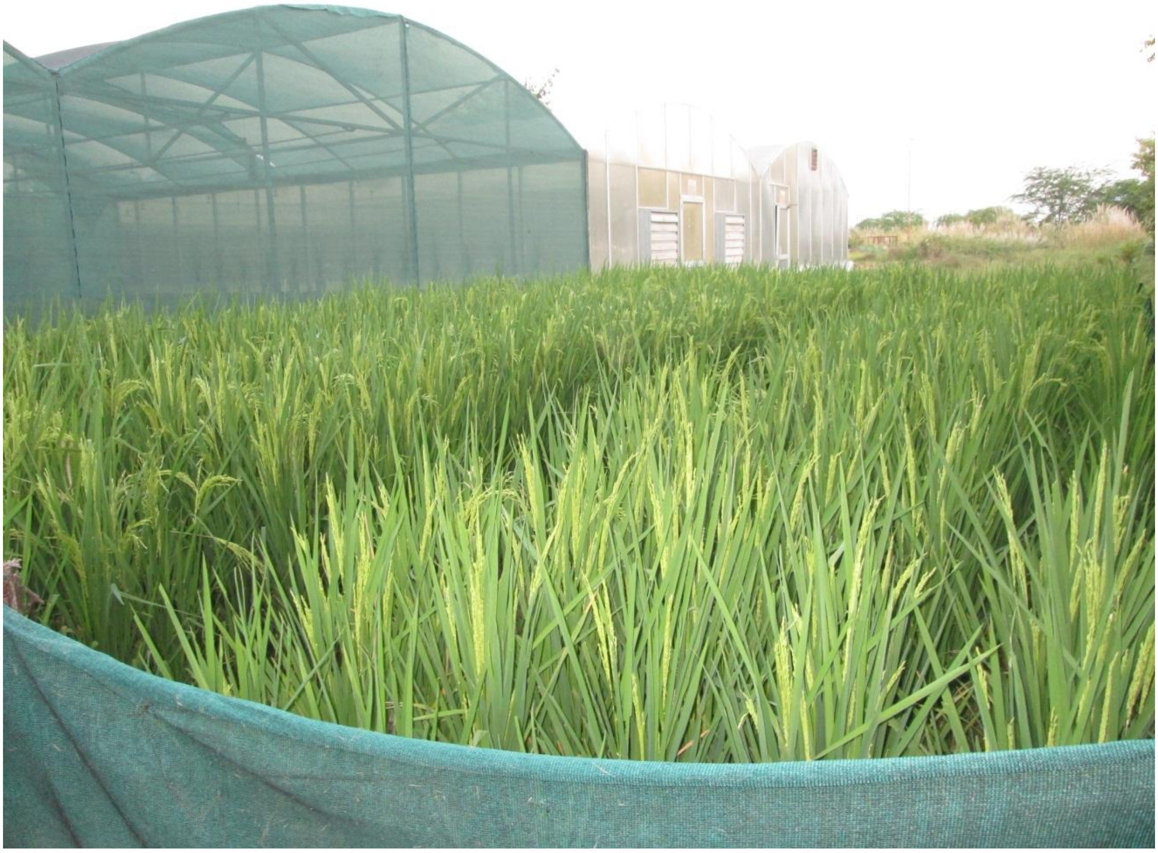

Figure 3: Experimental field station at BBA University, Lucknow