# Enhanced Growth and Yield of Rice (*Oryza sativa* L.) and Soil Enrichment are Mediated by Enhanced Availability of N and P in Soil and Plant Leaves on Application of Organic Matrix Entrapped Urea and DAP

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### *Abstract*

Field experiments were conducted during the Kharif season of the cropping years 2011 and 2012 at the Experimental Field Station of Department of Environmental Science, Babasaheb Bhimrao Ambedkar University, Lucknow to compare the efficacy of organic matrix entrapped chemical fertilizers i.e. Urea and DAP (OMECF) with the conventional chemical fertilizers (CCF) and no fertilizer (NF) for the cultivation of rice (*Oryza sativa*). The OMECF prepared in granular form contained cow dung, powder of neem leaves (*Azadirachta indica*), and clay soil (diameter of particles < 0.02 mm) in 1:1:1 ratios and 15% saresh (plant gum of *Acacia* sp.) as binder a long with half and one fourth of the recommended dose of commercially available urea and DAP. Single basal application of OMECF showed an increase in plant growth in terms of fresh and dry weights, root length, shoot length, root and leaves numbers and levels of $NO_3^-$, $NO_2^-$, $NH_4^+$ and $PO_4^-$in the rhizosphere and their accumulation in plant leaves.

***Key words:*** Organic matrix entrapped chemical fertilizer, *Oryza sativa*, *Azadirachta indica*, Saresh.

## 1. Introduction

Rice is one of the world's most important cereal crops with high agricultural and economic importance and considered as a staple food for more than 50% population worldwide. Asian farmers produce more than 90% of rice dominated by India and China, growing more than half of the total rice production (IRRI, 2011). Green Revolution has helped the global rice production to increase from 34.58 million tonnes in the year 1961 to 99.18 million tonnes in the year 2011 (FAI, 2012). However, the elevated consumption of chemical fertilizers to grow rice in green revolution package has created adverse environmental impacts.

About 15% of the total nitrogenous fertilizers used in agriculture are being applied to the rice system alone (Heffer, 2009). A specific problem with nitrogen (N) management in lowland rice ecosystems is the poor nitrogenuse efficiencies (NUE). Poor NUE for nitrogenous fertilizers results loss of the nutrients through various mechanisms such as volatilization, leaching and denitrification (Xiang *et al*., 2008; Zou *et al*., 2009; Kiran *et al*., 2010; Soares *et al*., 2012; Xu *et al*., 2012). Therefore, it is important to search for alternatives nitrogen fertilizers which can reduce nitrogen losses, improving nitrogen efficiency.

A number of alternatives have been adopted during the last decades, e.g. biofertilizers, integrated plant nutrient system (IPNS), and farm yard manure (FYM) (Singh *et al*., 2008, 2010; Cong *et al*., 2011), however, due to the limitations of availability and efficacy for these alternatives, their use by the farmers is limited and these alternatives are unable to replace the use of conventional chemical fertilizers.

In addition, slow/controlled release fertilizers (SRF) have also been used to supply nutrients at a rate that needed by the crop. These new generation fertilizers reduce the nutrient losses. The application of slow release fertilizers requires a reduced fertilizer dose and, consequently, reduced nutrient loading in the soil. The need of slow release fertilizers in the developing country like India is more urgent, where agricultural practices are very extensive and continuous. An effective saving of chemical fertilizer is directly correlated to the savings in the foreign currency and energy input consumed in the production of chemical fertilizers. Several slow release fertilizers have already been tested for rice (Carreres *et al*., 2003; Dahiya *et al*., 2004; Kondo *et al*., 2005; Singh *et al*., 2007; Tang *et al*., 2007; Kiran *et al*., 2010; Patil *et al*., 2010; Kumar *et al*., 2012), however, the economy and efficacy of the fertilizers need a constant revisit to design the fertilizers as per the specific needs of a crop so as to achieve maximum improved productivity.

Here, we have developed a low-cost, highly efficient, sustainable organic matrix entrapped slow release fertilizer, using local biodegradable agro waste like cow dung, clay soil, neem leaves easily available for local production by the small-scale industries or by the farmers. In our previous studies we have demonstrated that lesser urea entrapped in organic matrix containing cow dung, clay soil, neem leaf powder, rice bran, and polyvinyl alcohol (PVA) as a carrier prepared in the form of super granules enhances growth, productivity, and yield in rice (Dahiya *et al*., 2004). In present study, costly polyvinyl alcohol (PAV) has been replaced with a cheaper binder saresh (acacia gum) and a new slow release organic matrix entrapped chemical fertilizer (OMECF) has been developed. The objective of this paper is to investigate the response of OMECF on availability of inorganic N species (nitrate, nitrite and ammonium) and phosphate in rhizosphere and in rice leaves and to evaluate a correlation of these nutrients with productivity and yield of rice.

## 2. Materials and Methods

### *2.1. Experimental site*

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$^o$ and 27.10$^o$ North latitude and 80.30$^o$ and 81.13$^o$ East longitude. The certified seeds of rice plant [*Oryza sativa* L. cv. Moti (IET-7328)] was obtained from a local dealer of Lucknow.

### *2.2. Field experimental design*

The experimental design was a randomized block of seven treatments replicated three times with two determinations each. The plot size was 1.5 x 1.5 m seven treatments were designed as

NF = no fertilizer, FOM = free form of organic matrix, EOM = entrapped form of organic matrix, CCF-I=free form of 1/2 recommended dose (RD) of urea (75 kg ha$^{-1}$) and DAP (30kg ha$^{-1}$), OMECF-I= entrapped form of 1/4 RD of urea (37.5 kg ha$^{-1}$) and DAP (15kg ha$^{-1}$) CCF-II= free form of R D of urea (150 kg ha$^{-1}$) and DAP (60 kg ha$^{-1}$), OMECF-II=entrapped form of 1/2 RD of urea (75 kg ha$^{-1}$) and DAP (30 kg ha$^{-1}$)

### *2.3. Preparation of organic matrix entrapped slow release granules*

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 oven at 60-70$^o$C for 3 days and powdered in a grinder and mixer. These supporting matrixes were mixed in 1:1:1 ratio. These matrixes were mixed with 15% commercial saresh (plant gum of *Acacia*) and small granules of approximately 5 mm diameter were prepared manually and dried at room temperature. In the same way, the organic matrix entrapped slow release granules of chemical fertilizers were also prepared taking ¼ and ½ RD of urea and DAP.

### *2.4. 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 i.e. root and shoot length, number of roots and leaves 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 rhizosphere soil from the different places of the experimental plots. Soil pH was measured electrometrically using glass electrode pH meter, Jackson (1967). Electrical conductivity was measured by the method of Richards (1954). Organic carbon in the soil samples were estimated using the method described by Walkley and Black (1934). Available nitrogen in soil was estimated using the alkaline potassium permanganate by the method described earlier, Subbaih and Asija (1955). Available phosphorus in soil was estimated by the method of Olsen *et al*. (1954). Soluble potassium was also estimated by the method described by Jackson (1958).

Soil layer, dried by venting, sieved and stored in loosely tied plastic bags to ensure sufficient aeration and prevent moisture loss prior to assaying for nitrate, nitrite, ammonium and phosphate content.

Nitrate content in soil and leaves were estimated by the method described by Cataladu *et al*. (1975), by using 5% salicylic acid solution in concentrated sulphuric acid and 2N sodium hydroxide. Nitrite content in soil and leaves were estimated by the method described by Steven and Oaks (1973), usinghomogenate of the sample with sulphanilamide and N- (1- Naphthyl) - ethylene - diamine dihydrochloride. Ammonium content in soil and leaves were estimated by the method described by Weatherburn (1967), using Nessler's reagent. Phosphate content in soil and leaves were estimated by the stannous chloride methodusing ammonium molybedate and $SnCl_2$. Absorbance of the solutions were recorded at 410, 540, 420 and 680 nm for nitrate, nitrite, ammonium and phosphate respectively using UV-visible spectrophotometer (Varian, carry 100 Bio). Protein content in leaves was estimated by Lowry *et al*. (1951) method, using bovine serum albumin (BSA) as standard.

Microbial biomass was measured by plate count method described by APHA (1984).

### *2.5. 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 multirange test are presented in the figures and tables as the mean ±SD (n=6).

## 3. Results

### *3.1. Growth parameters*

The organic matrix entrapped chemical fertilizers (OMECFs) enhanced various growth parameters over free forms of chemical fertilizers and no fertilizer at different growth stages of rice plants. The organic matrix entrapped form of chemical fertilizers (OMECF-I and OMECF-II) showed higher root biomass among all the fertilizer application. Root length and number of roots were significantly increased with the application of OMECFs over CCFs and NF.

Fresh weight of roots of rice plant applied with the application of OMECF-I increased by 75 and 19.0% over NF and CCF-I whereas in case of OMECF-II it was 82 and 9.8% over NF and CCF-II, respectively. In the case of dry weight of roots it was 32 and 10% over NF and CCF-I and 68 and 22.6% over NF and CCF-II, respectively (Table 1).

**Table 1:** Effect of different fertilizer applications on the root length, number of roots, fresh and dry biomass of roots of rice plant on 30, 60, 90 and 120 DAT

| | **Treatments** | **30d** | **60d** | **90d** | **120d** |
|---|---|---|---|---|---|
| **Root length (cm)** | NF | 5.70±0.10$^{e}$ | 8.30±0.20$^{d}$ | 8.53±0.12$^{e}$ | 9.57±0.32$^{e}$ |
| | FOM | 6.37±0.21$^{d}$ | 9.37±0.15$^{c}$ | 10.07±0.12$^{d}$ | 12.77±0.15$^{d}$ |
| | EOM | 6.57±0.15$^{cd}$ | 9.80±0.26$^{b}$ | 10.50±0.15$^{c}$ | 13.40±0.30$^{c}$ |
| | CCF-I | 6.87±0.25$^{bc}$ | 10.57±0.25$^{a}$ | 11.37±0.21$^{b}$ | 14.40±0.20$^{b}$ |
| | OMECF-I | 7.17±0.21$^{ab}$ | 10.63±0.15$^{a}$ | 11.73±0.12$^{a}$ | 14.60±0.06$^{a}$ |
| | CCF-II | 7.43±0.21$^{a}$ | 10.77±0.06$^{a}$ | 11.53±0.15$^{ab}$ | 14.63±0.15$^{ab}$ |
| | OMECF-II | 7.50±0.20$^{a}$ | 10.83±0.15$^{a}$ | 11.77±0.06$^{a}$ | 14.77±0.17$^{a}$ |
| **Number of roots** | NF | 29.67±1.53$^{e}$ | 36.00±1.00$^{f}$ | 44.67±1.53$^{e}$ | 53.67±1.53$^{f}$ |
| | FOM | 33.00±1.73$^{d}$ | 39.00±1.00$^{e}$ | 48.67±2.08$^{d}$ | 70.33±2.08$^{e}$ |
| | EOM | 33.67±1.53$^{d}$ | 43.00±2.00$^{d}$ | 52.67±1.53$^{c}$ | 72.67±1.53$^{de}$ |
| | CCF-I | 38.00±1.00$^{c}$ | 57.33±1.53$^{c}$ | 73.00±2.00$^{b}$ | 75.00±2.65$^{cd}$ |
| | OMECF-I | 39.67±1.53$^{bc}$ | 62.67±1.53$^{ab}$ | 73.33±1.53$^{b}$ | 77.00±1.00$^{bc}$ |
| | CCF-II | 41.00±1.00$^{ab}$ | 60.33±2.52$^{b}$ | 75.00±2.65$^{ab}$ | 78.67±1.15$^{ab}$ |
| | OMECF-II | 42.33±1.53$^{a}$ | 64.33±0.58$^{a}$ | 77.00±1.00$^{a}$ | 81.67±2.08$^{a}$ |
| **Fresh weight of roots (g)** | NF | 0.68±0.03$^{c}$ | 1.03±0.02$^{d}$ | 4.16±0.19$^{d}$ | 9.86±0.12$^{d}$ |
| | FOM | 0.88±0.04$^{b}$ | 1.32±0.06$^{c}$ | 5.66±0.21$^{c}$ | 11.48±0.27$^{c}$ |
| | EOM | 0.90± 0.01$^{b}$ | 1.37±0.03$^{b}$ | 5.84±0.10$^{b}$ | 11.89±0.02$^{b}$ |
| | CCF-I | 0.90±0.02$^{b}$ | 1.54±0.21$^{b}$ | 6.54±0.24$^{b}$ | 14.52±1.37$^{b}$ |
| | OMECF-I | 0.95±0.03$^{a}$ | 1.77±0.09$^{a}$ | 6.90±0.03$^{a}$ | 17.30±1.47$^{a}$ |
| | CCF-II | 0.96±0.04$^{a}$ | 1.58±0.05$^{ab}$ | 6.88±0.05$^{ab}$ | 16.22±1.06$^{b}$ |
| | OMECF-II | 0.97±0.02$^{a}$ | 1.82±0.04$^{a}$ | 6.97±0.06$^{a}$ | 18.0±0.94$^{a}$ |
| **Dry weight of roots (g)** | NF | 0.04±0.01$^{d}$ | 0.11±0.02$^{e}$ | 0.13±0.04$^{e}$ | 0.74±0.06$^{e}$ |
| | FOM | 0.06±0.02$^{c}$ | 0.21±0.02$^{d}$ | 0.21±0.03$^{d}$ | 0.85±0.04$^{d}$ |
| | EOM | 0.07±0.02$^{bc}$ | 0.31±0.16$^{bc}$ | 0.24±0.04$^{bc}$ | 0.90±0.03$^{bc}$ |
| | CCF-I | 0.09±0.01$^{ab}$ | 0.31±0.05$^{b}$ | 0.31±0.04$^{b}$ | 0.96±0.01$^{b}$ |
| | OMECF-I | 0.10±0.01$^{a}$ | 0.27±0.02$^{ab}$ | 0.35±0.02$^{ab}$ | 0.98±0.01$^{ab}$ |
| | CCF-II | 0.11±0.01$^{a}$ | 0.27±0.03$^{b}$ | 0.35±0.03$^{b}$ | 1.02±0.03$^{b}$ |
| | OMECF-II | 0.12±0.03$^{a}$ | 0.39±0.01$^{a}$ | 0.38±0.01$^{a}$ | 1.25±0.17$^{a}$ |

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, CCF-I=free form of 1/2 recommended dose (RD) of urea (75 kg ha$^{-1}$) and DAP (30 kg ha$^{-1}$), OMECF-I= entrapped form of 1/4 RD of urea (37.5 kg ha$^{-1}$) and DAP (15 kg ha$^{-1}$), CCF-II= free form of RD of urea (150 kg ha$^{-1}$) and DAP (60 kg ha$^{-1}$), OMECF-II=entrapped form of 1/2 RD of urea (75 kg ha$^{-1}$) and DAP (30 kg ha$^{-1}$)

**Table 2:** Effect of different fertilizer applications on the shoot length, number of leaves, fresh and dry biomass of shoots of rice plant on 30, 60, 90 and 120 DAT

| | Treatments | 30d | 60d | 90d | 120d |
|---|---|---|---|---|---|
| Shoot length (cm) | NF | 37.73±1.50$^{d}$ | 46.03±0.93$^{e}$ | 53.63±0.40$^{f}$ | 74.07±0.50$^{g}$ |
| | FOM | 40.00±0.20$^{c}$ | 61.70±1.49$^{d}$ | 74.27±0.35$^{e}$ | 96.33±1.22$^{f}$ |
| | EOM | 40.57±0.31$^{bc}$ | 64.83±1.26$^{c}$ | 76.73±0.21$^{d}$ | 100.33±1.36$^{e}$ |
| | CCF-I | 41.13±0.21$^{ab}$ | 72.13±0.81$^{b}$ | 85.50±0.26$^{c}$ | 108.87±1.03$^{d}$ |
| | OMECF-I | 41.50±0.30$^{ab}$ | 75.0±0.60$^{a}$ | 86.07±0.21$^{c}$ | 118.13±0.50$^{b}$ |
| | CCF-II | 41.37±0.21$^{ab}$ | 76.23±0.59$^{b}$ | 88.00±0.87$^{b}$ | 116.57±0.67$^{c}$ |
| | OMECF-II | 41.77±0.15$^{a}$ | 78.77±0.57$^{a}$ | 89.50±0.78$^{a}$ | 119.87±0.32$^{a}$ |
| Number of leaves | NF | 5.67±0.58$^{c}$ | 6.67±0.58$^{e}$ | 10.33±0.58$^{f}$ | 9.67±0.58$^{e}$ |
| | FOM | 7.00±1.00$^{bc}$ | 10.33±1.53$^{d}$ | 13.33±0.58$^{e}$ | 12.67±0.58$^{d}$ |
| | EOM | 8.33±0.58$^{ab}$ | 11.00±1.00$^{c}$ | 15.67±0.58$^{d}$ | 15.67±0.58$^{c}$ |
| | CCF-I | 9.00±1.00$^{a}$ | 12.33±0.58$^{bc}$ | 16.67±0.58$^{c}$ | 16.67±0.58$^{bc}$ |
| | OMECF-I | 9.00±1.00$^{a}$ | 13.33±1.15$^{b}$ | 19.00±1.15$^{b}$ | 19.00±0.00$^{a}$ |
| | CCF-II | 9.33±0.58$^{a}$ | 13.67±0.58$^{b}$ | 18.33±0.58$^{b}$ | 17.33±1.15$^{b}$ |
| | OMECF-II | 9.67±0.58$^{a}$ | 15.67±0.58$^{a}$ | 20.33±0.58$^{a}$ | 19.33±1.15$^{a}$ |
| Fresh weight of shoot (g) | NF | 2.76±0.06$^{d}$ | 4.61±0.66$^{d}$ | 11.33±0.58$^{f}$ | 46.67±2.89$^{e}$ |
| | FOM | 3.79±0.21$^{c}$ | 8.67±0.42$^{a}$ | 15.64±0.56$^{e}$ | 76.67±3.88$^{d}$ |
| | EOM | 3.92±0.15$^{b}$ | 8.90±0.44$^{a}$ | 17.95±0.28$^{d}$ | 78.33±2.81$^{d}$ |
| | CCF-I | 3.97±0.11$^{bc}$ | 8.97±0.06$^{a}$ | 27.10±1.25$^{c}$ | 91.67±3.89$^{c}$ |
| | OMECF-I | 4.08±0.07$^{ab}$ | 9.04±0.82$^{c}$ | 32.45±2.16$^{b}$ | 93.33±3.41$^{bc}$ |
| | CCF-II | 4.13±0.25$^{a}$ | 9.28±0.11$^{a}$ | 33.07±0.85$^{b}$ | 96.67±3.81$^{b}$ |
| | OMECF-II | 4.23±0.11$^{a}$ | 9.58±0.65$^{b}$ | 38.66±1.66$^{a}$ | 103.33±5.21$^{a}$ |
| Dry weight of shoot (g) | NF | 0.64 ± 0.07$^{c}$ | 0.90±0.03$^{c}$ | 2.34±0.11$^{d}$ | 8.38±0.06$^{g}$ |
| | FOM | 0.86±0.03$^{b}$ | 1.34±0.01$^{b}$ | 3.58±0.16$^{c}$ | 9.82±0.12$^{f}$ |
| | EOM | 0.90±0.02$^{ab}$ | 1.38±0.04$^{b}$ | 4.44±0.04$^{b}$ | 10.59±0.22$^{e}$ |
| | CCF-I | 0.93±0.05$^{a}$ | 1.67±0.03$^{ab}$ | 4.85±0.06$^{a}$ | 11.91±0.07$^{d}$ |
| | OMECF-I | 0.95±0.02$^{a}$ | 1.89±0.05$^{a}$ | 4.89±0.01$^{a}$ | 13.89±0.11$^{c}$ |
| | CCF-II | 0.96±0.01$^{a}$ | 1.92±0.05$^{a}$ | 4.92±0.06$^{a}$ | 15.25±0.54$^{b}$ |
| | OMECF-II | 0.97±0.02$^{a}$ | 1.95±0.52$^{a}$ | 4.95±0.04$^{a}$ | 18.40±0.53$^{a}$ |

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, CCF-I=free form of 1/2 Recommended dose (RD) of urea (75 kg ha$^{-1}$) and DAP (30 kg ha$^{-1}$), OMECF-I= entrapped form of 1/4 RD of urea (37.5 kg ha$^{-1}$) and DAP (15 kg ha$^{-1}$), CCF-II= free form of RD of urea (150 kg ha$^{-1}$) and DAP (60 kg ha$^{-1}$), OMECF-II=entrapped form of 1/2 RD of urea (75 kg ha$^{-1}$) and DAP (30 kg ha$^{-1}$)

Shoots length, number of leaves as well as fresh and dry weights of shoots were also significantly increased during the crop growing up to the time of harvest by the application of OMECF-I and OMECF-II over the CCFs. The entrapped fertilizers caused more significant increase in shoot length than that of FCFs and NF. Number of leaves increased by 99 and 96% over NF with the application OMECF-I and OMECF-II. Fresh weight of shoots of rice plant applied with the application of OMECF-I increased by 99 and 4.7% over NF and CCF-I whereas in case of OMECF-II, it was 121 and 6.7% over NF and CCF-II, respectively. In the case of dry weight of shoots it was 65 and 119% over NF and 16.1 and 20.9% over CCF-I and CCF-II, respectively (Table 2).

Both the grain yield and straw yield of rice was affected by application of different types of the fertilizers (Fig. 1). Application of OMECFs increased grain and straw yield over the application of CCFs. The percentage increase of 9.21 and 71% in grain yield was recorded in harvesting stage by the application of OMECF-I over the CCF-I and NF and 11.17, 25.12 and 76.40% increase was recorded in presence of OMECF-II over CCF-II, CCF-I and NF. The straw yield increased by 8.04% in OMECF-I over CCF-I and 9.68% in OMECF-II over CCF-II (Fig. 1). In this study the highest grain yield (3.94 t ha$^{-1}$) and straw yield (4.65 t ha$^{-1}$) was observed from OMECF-II (Fig. 1). The free form of organic matrix (FOM) and its entrapped form (EOM) significantly affects the growth of rice over NF but less effective than CCFs and OMECFs. CCF-II, yet caused higher plant growth over CCF-I, difference between the two is difficult to explain the application of double amount of chemical fertilizers as recommended dose (CCF-II) over its half dose (CCF-I). There was no significant difference between OMECF-II and CCF-II. OMECF-II having only half of the recommended dose of urea and DAP produced almost same results as the full recommended dose of soluble urea and DAP alone.

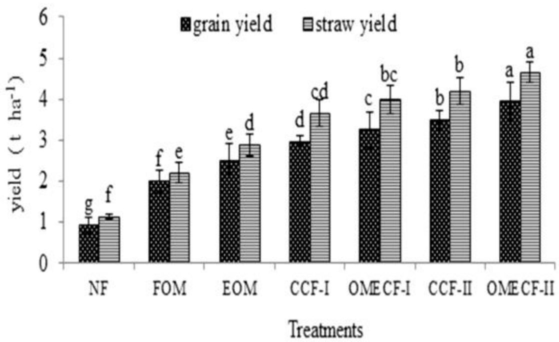


**Fig. 1**: Effect of different fertilizer applications on grain and straw yield (t $ha^{-1}$) of rice plant. Values are means ±SD, (one way ANOVA) DMRT significant at $p<0.05$, followed by the same letter(s) are not significantly different at $p<0.05$. Where, NF = no fertilizer, FOM= free form of organic matrix, EOM= entrapped form of organic matrix, CCF-I=free form of 1/2 recommended dose (RD) of urea (75 kg $ha^{-1}$) and DAP (30 kg $ha^{-1}$), OMECF-I= entrapped form of 1/4 RD of urea (37.5 kg $ha^{-1}$) and DAP (15 kg $ha^{-1}$), CCF-II= free form of RD of urea (150 kg $ha^{-1}$) and DAP (60 kg $ha^{-1}$), OMECF-II=entrapped form of 1/2 RD of urea (75 kg $ha^{-1}$) and DAP (30 kg $ha^{-1}$)

### *3.2 Level of nitrate, nitrite, ammonium and phosphate content in soil and leaves of rice plant*

Nitrate, nitrite, ammonium and phosphate content in soil and leaves of rice plant were also influenced by different fertilizer application methods. The organic matrix entrapped chemical fertilizer increased nitrate, nitrite, ammonium and phosphate content at different growth stages.

Nitrate content in rice leaves applied with the application of OMECF-II increased by 18.07, 16.76 and 17.5% over CCF-II and 117.77, 108.24 and 85.52% over NF on 60. 90 and 120 DAT, respectively. OMECF-I also increased nitrate content in leaves over CCF-I and NF (Fig. 2B). The soil nitrate increased by 20.59, 20.60 and 32.78% respectively on 60, 90 and 120 DAT with application of the OMECF-I over CCF-I and 192.85, 210 and 223.98% over NF. The percentage increase of nitrate in soil of the experimental field was 13.51, 12.04 and 19.44% respectively on 60, 90 and 120 DAT with OMECF-II over CCF-II and 199.98, 220.70 and 243.98% over NF (Fig. 2A).

Nitrite content in rice leaves applied with OMECF-I also increased by 14.85, 3.40 and 4.51% respectively on 60, 90 and 120 DAT respectively over CCF-I and 156.71, 91 and 102.78% over NF. OMECF-II also increased nitrite content in leaves by 17.4, 5.21 and 6.18% over CCF-II and 166.41, 112.65 and 102.78% at different growth stages (Fig. 3B). The application of OMECF-I and OMECF-II also increased soil nitrite significantly at 60, 90 and 120 DAT respectively over NF and CCFs (Fig. 3A).

The ammonium content in rice leaves increased by 4.26, 8.1 and 7.19% respectively on 60, 90 and 120 DAT with application of the OMECF-II over CCF-II and 55.19, 64.56, 93.03 and 139.10% over NF on 30, 60, 90 and 120 DAT. The percentage increase of nitrate was 10.47, 4.70 and 12.99% respectively on 60, 90 and 120 DAT with OMECF-I over CCF-I and 41.84, 61.74, 85.12 and 123.73% over NF (Fig. 4B). Ammonium content in rice soil applied with the application of OMECF-II increased by 15.67, 15.15 and 11.37% on 60, 90 and 120 DAT respectively over CCF-II. OMECF-I also increased ammonium content in soil over CCF-I (Fig. 4A).

Phosphate content in fresh leaves of rice plants were significantly increased in treatment of OMECFs (½ and ¼ recommended dose of Urea and DAP) in compare to NF and CCFs (Fig. 5B). The application of OMECF-I and OMECF-II also increased soil phosphate at 60, 90 and 120 DAT respectively over NF and CCFs (Fig. 5A). OMECFs increased the levels of $NO_3^-$, $NO_2^-$, $NH_4^+$and $PO_4^-$ in the plant's rhizosphere (0-15 cm) and its mobilization from soil to the plant leaves, which seem to be correlated to the growth and biomass accumulation in rice. Correlation figures shows that the correlation between average soil nitrate, nitrite, ammonium, phosphate and average plant nitrate, nitrite, ammonium, phosphate at 120 days in different treatments are linearly significant (Fig. 2C, 3C, 4C and 5C).

### *3.3. Level of protein content in rice leaves*

The organic matrix entrapped chemical fertilizer increased protein content in leaves at different day's interval. Protein content was increased by 5.75, 4.83 and 8.74% at 60, 90 and 120 DAT with application of the OMECF-II over CCF-II and 132, 119 and 118% over NF, respectively. OMECF-I also increased protein content by 11.7, 9.61 and 6.74% over CCF-I and 98.7, 92.13 and 91.20% over NF respectively at 60, 90 and 120 DAT (Fig. 6).

### *3.4 Changes in physic-chemical and biological properties of soil*

Soil physico-chemical properties were significantly influenced with the application of fertilizers (Table 3). Application of OMECF increased % OC, total and available N, available P and K over CCF and NF. Single basal application of OMECF showed increase in total N, available N, available P and soluble K over other treatments. It seems that the OMECF 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 OMECF significantly increased the soil microbes' population compared with CCF and NF (Table 4).

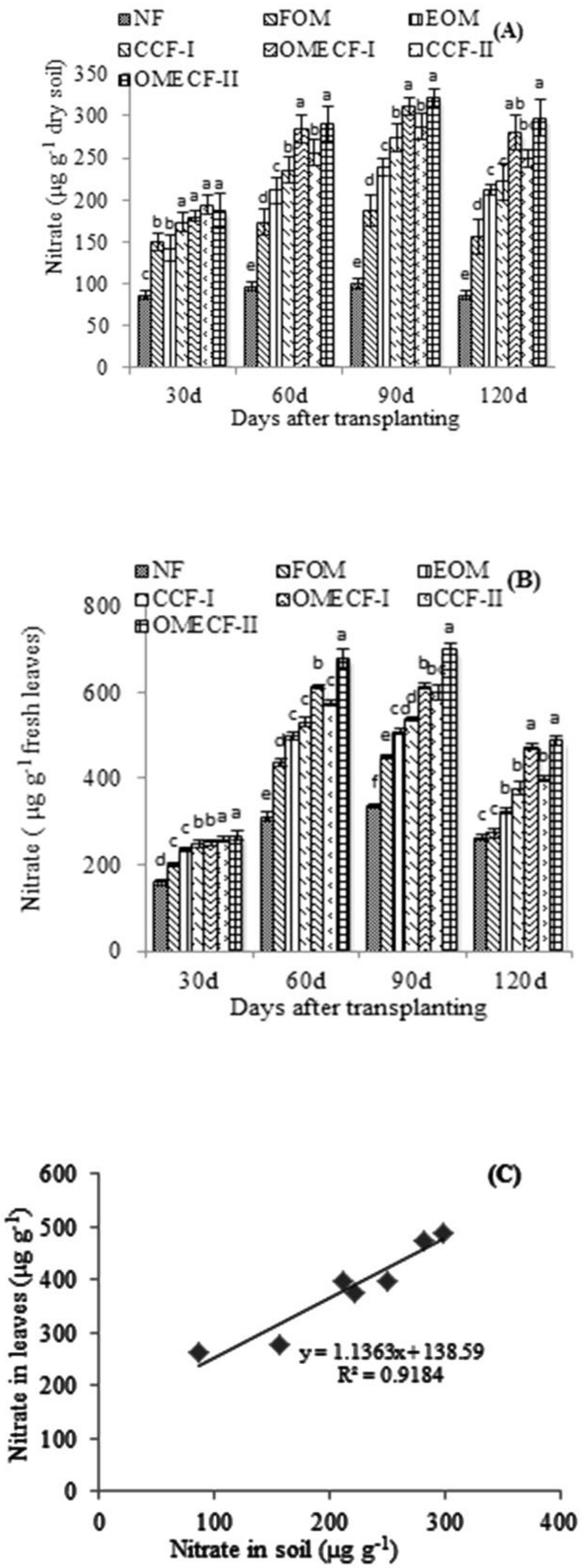


**Fig. 2:** Levels of Nitrate (µg $g^{-1}$) in dry soil (A) and leaves (B) of rice plant at 30, 60, 90 and 120 DAT and correlation between average soil nitrate and average plant nitrate (C) at120 days in different treatments (as defined in Fig. 1)

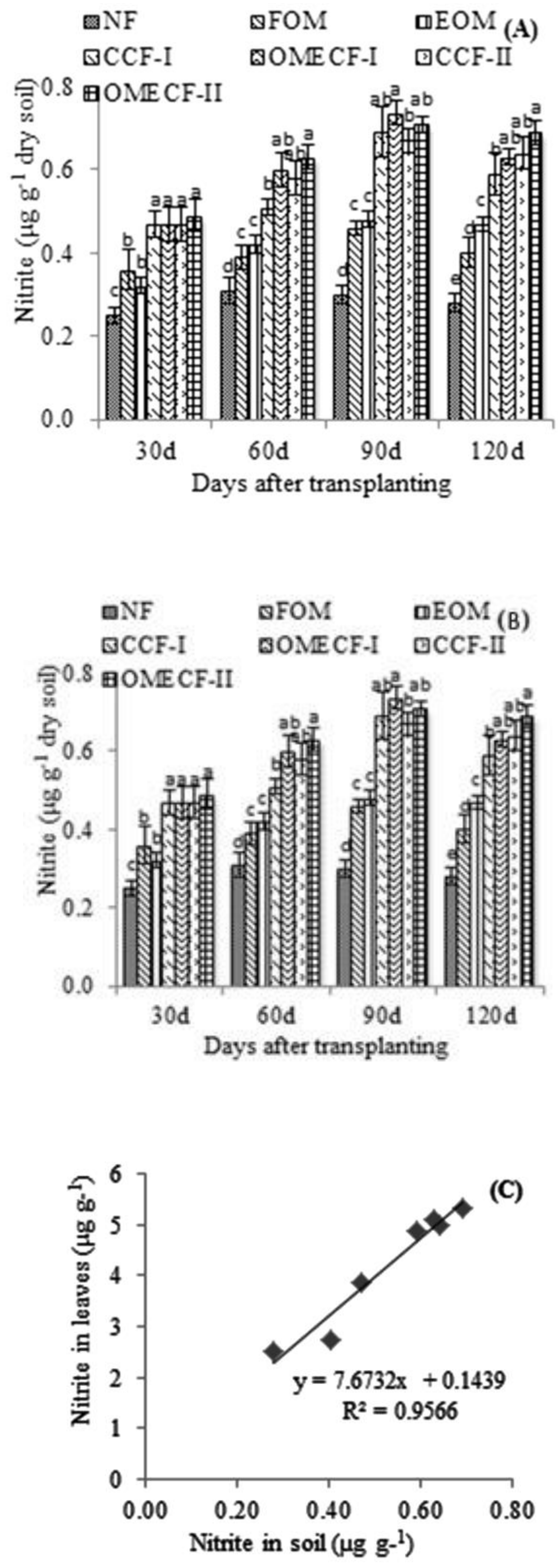


**Fig. 3:** Levels of nitrite (µg $g^{-1}$) in dry soil (A) and leaves (B) of rice plant at 30, 60, 90 and 120 DAT and correlation between average soil nitrite and average plant nitrite (C) at 120 days in different treatments (as defined in Fig. 1)

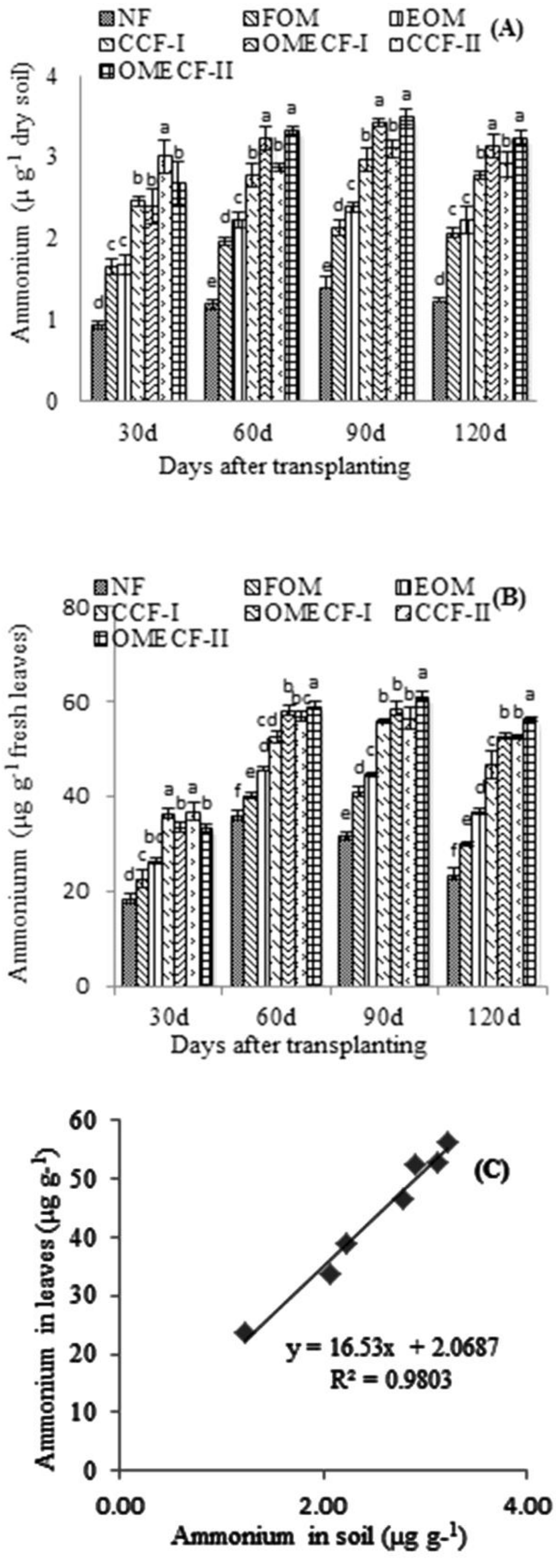


**Fig. 4:** Levels of ammonium (µg $g^{-1}$) in dry soil (A) and leaves (B) of rice plant at 30, 60, 90 and 120 DAT and correlation between average soil ammonium and average plant ammonium at 120 DAT in different treatments (as defined in Fig. 1)

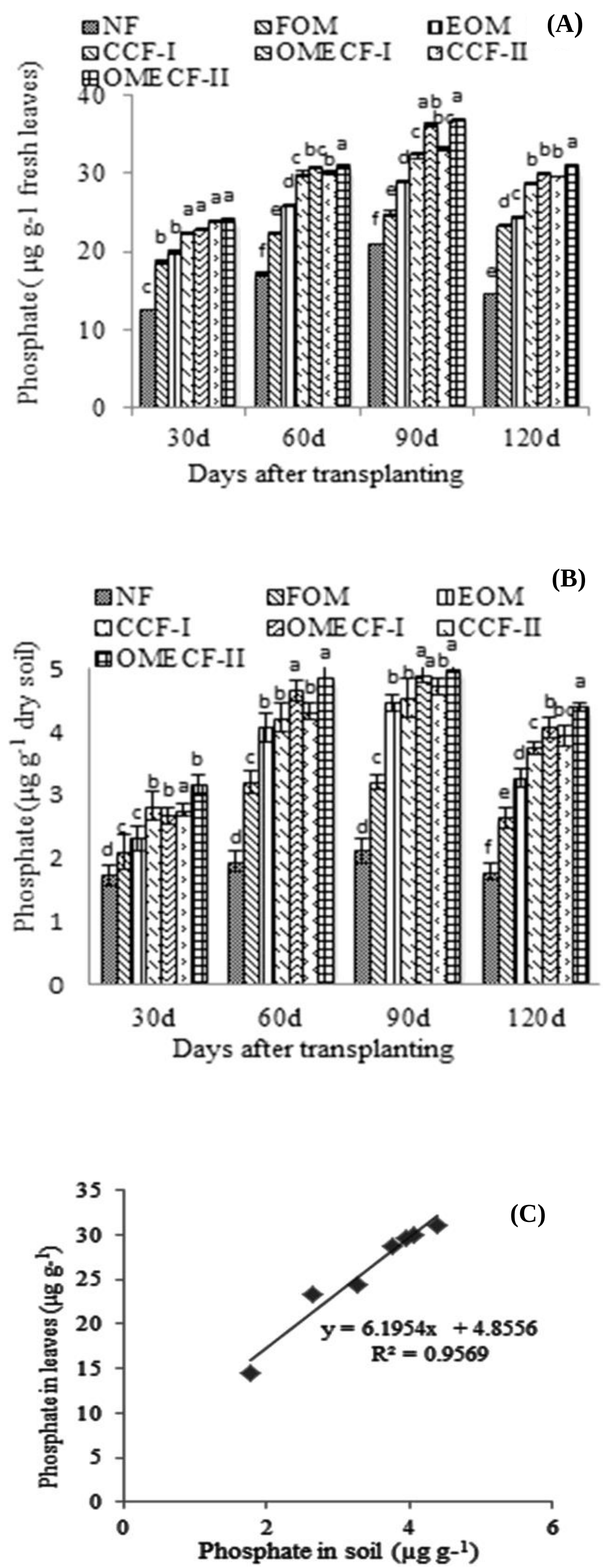


**Fig. 5:** Levels of phosphate (µg $g^{-1}$) in dry soil (A) and leaves (B) of rice plant at 30, 60, 90 and 120 DAT and correlation between average soil phosphate and average plant phosphate at 120 DAT in different treatments (as defined in Fig. 1)

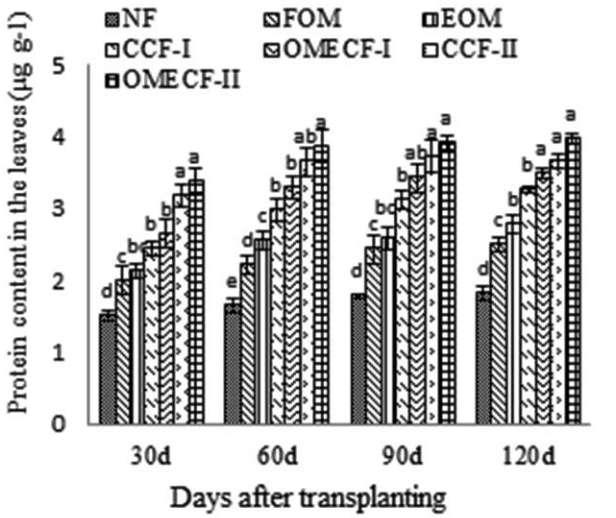


**Fig. 6**: Levels of protein (µg $g^{-1}$) in leaves of rice plant at 30, 60, 90 and 120 DAT in different treatments (as defined in Fig. 1)

## 4. Discussion

Fertilizer nutrient input, especially nitrogen (N) fertilizer, plays an important role in increasing crop yields. To maximize grain yield, farmers often apply a much higher amount of nitrogen fertilizer than the crops needed. High N inputs always result in low N use efficiency and serious N losses (Wang *et al*., 2001; Peng *et al*., 2006), and consequently lead to serious pollutants of surface water, ground-water, and atmosphere (Ju *et al*., 2009). Paddy rice is one of the most important cereal crops in Monsoon in Asia (Kyuma, 2004). Nitrogen use efficiency is relatively low in irrigated rice because of rapid N losses through ammonia volatilization, denitrification, surface runoff and leaching. The yield plateau of rice and adverse environmental impacts due to imbalance use of chemical fertilizers illustrate global attention for effective fertilizer management. Therefore, efficient fertilizer management under environment friendly condition is crucial to increase rice production worldwide (Naher *et al*., 2011).

Slow release fertilizers have been recognized as a sustainable and better solution for various environmental problems caused by traditional conventional chemical fertilizers. With SRFs, dosage requirements are lowered, fertilizer use efficiency is improved and environmental problems are practically negligible. Application of SRFs enhances efficiency of fertilizers, reduces fertilizer loss and improves yield and quality of crops (Kiran *et al*., 2010).

However, most of the commercial slow-release fertilizers are expensive and still away from the ordinary farmers in the developing countries. Therefore, we have developed granular slow-release fertilizers using local biodegradable, non-toxic, eco-friendly and low-cost agro-waste materials which have increased the efficacy of nutrients very significantly for rice, wheat and Indian mustard crops (Singh *et al*., 2006; Sharma and Singh, 2011; Sharma *et al*., 2011; Kumar *et al*., 2012, 2013a, b, 2014). A similar formulation using entrapment of urea in such organic matrix has been demonstrated earlier by our own group for enhanced productivity and yield of rice (Dahiya *et al*., 2004; Kumar *et al*., 2012), however, the binder used in the earlier study was polyvinyl alcohol (PVA) which is much more expensive than the acacia gum used in this study and studies on nutrient availability and microbial enrichment in soil as well as its correlation with nutrient enrichment in plant tissues subsequently affecting growth and yield have not been reported earlier.

In this case, OMECFs has proved a better source of NPK and enhanced microbial population in the soil, hence built up a more favourable nutritional environment during growth and development of rice. A large root system results from good nutritional environment of the soil transfer more nutrients to the plants which is reflected as higher growth of shoots. It has been observed that single basal application of SRFs significantly increased root length and root biomass (Tang *et al*., 2007). This indicates that SRFs could greatly promote the growth and development of root system, particularly at mid (60 DAT) and late (120 DAT) growth stages, thus enlarging the spaces of nutrient absorption. The OMECFs strengthened the transportation of nutrients from root system to aboveground parts of a crop. Therefore, a large root system with deep and extensive distribution was able to establish a solid foundation for forming a high yielding plant. In this study, single basal application of OMECFs contribute greater supply of soil available N over CCFs and NF and thus, creating a suitable nutritional environment for vigorous growth of rice.

A very significant increase in shoot length, shoot biomass in rice was recorded at 60 and 120 days after application of OMECFs over CCFs and NF plots. OMECFs are expected to supply N and P throughout the growing season. Therefore N and P ion (i.e. $NO_3^-$, $NO_2^-$, $NH_4^+$and $PO_4^+$) concentrations were higher in OMECFs treated plants. Increase in soil nutrients enhances soil fertility in terms of activity of microbial enzymes and microbial population in soil. Application of OMECF increased microbe population in soil as a consequence of enhanced availability of NPK and organic carbon. The reasons might be that organic matrix supplied plentiful carbon sources which were required by soil microbes. Furthermore, the increase of organic substance in soils could change and improve the characteristics and the diversity of microbial metabolism in soil. Myrold *et al*. (2007) have suggested that the proportion of bacteria to fungi could reflect the soil fertility and higher proportion indicated higher soil fertility.

**Table 3:** 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 |
| CCF-I | 7.40 ± 0.05 | 0.21±0.06 | 0.55 ± 0.06 | 963.33±17.99 | 225.00±5.00 | 24.49 ±1.95 | 252.03±12.37 |
| OMECF-I | 8.06 ±0.13 | 0.22±0.03 | 0.61± 0.05 | 968.00±15.78 | 246.33±7.64 | 28.19 ± 1.34 | 257.36±15.84 |
| CCF-II | 7.76± 0.06 | 0.24±0.01 | 0.66± 0.06 | 971.0 ±15.01 | 253.33±8.32 | 30.88 ±2.36 | 278.21 ±17.25 |
| OMECF-II | 7.89 ±0.10 | 0.25±0.01 | 0.72± 0.06 | 978.0±10.02 | 258.00±8.77 | 35.80 ±2.19 | 284.00 ±17.76 |

Where, NF = no fertilizer, FOM= free form of organic matrix, EOM= entrapped form of organic matrix, CCF-I=free form of 1/2 Recommended dose (RD) of urea (75 kg $ha^{-1}$) and DAP (30 kg $ha^{-1}$), OMECF-I= entrapped form of 1/4 RD of urea (37.5 kg $ha^{-1}$) and DAP (15 kg $ha^{-1}$), CCF-II= free form of RD of urea (150 kg $ha^{-1}$) and DAP (60 kg $ha^{-1}$), OMECF-II=entrapped form of 1/2 RD of urea (75 kg $ha^{-1}$) and DAP (30 kg $ha^{-1}$)

**Table 4:** Effect of different fertilizer application on microbial properties 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 |
|---|---|---|
| Experimental field before transplanting of rice crop | 0.65 | 1.6 |
| Experimental field after harvesting of rice crop | | |
| NF | 1.0$^d$±0.00 | 2.0$^d$±1.00 |
| FOM | 1.3$^c$ ± 0.57 | 2.3$^d$±0.57 |
| EOM | 1.3$^c$± 0.57 | 2.6$^c$±0.57 |
| CCF-I | 1.6$^b$±0.57 | 3.0$^c$±1.00 |
| OMECF-I | 2.0$^a$± 1.00 | 3.6$^a$±0.57 |
| CCF-II | 1.6$^b$± 1.00 | 3.3$^b$±0.57 |
| OMECF-II | 2.3$^a$±1.15 | 4.3$^a$±0.57 |

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, CCF-I=free form of 1/2 Recommended dose (RD) of urea (75 kg $ha^{-1}$) and DAP (30 kg $ha^{-1}$), OMECF-I= entrapped form of 1/4 RD of urea (37.5 kg $ha^{-1}$) and DAP (15 kg $ha^{-1}$), CCF-II= free form of RD of urea (150 kg $ha^{-1}$) and DAP (60 kg $ha^{-1}$), OMECF-II=entrapped form of 1/2 RD of urea (75 kg $ha^{-1}$) and DAP (30 kg $ha^{-1}$))

These results suggested that the application of OMECFs caused better nutrient availability and their translocation to plants which subsequently caused higher growth and yield of rice. The data revealed that entrapment of commercially available conventional chemical fertilizers (urea and DAP) in the better carrier (organic matrix) can significantly increase the efficacy of urea and DAP for rice production as even half or one fourth doses of the fertilizers could enhance better productivity and yield in entrapped form. Organicmatrix proves to be a good binding matrix source when applied with chemical fertilizers.

Comparing many other reports associated with the application of slow-release fertilizers, controlled-release fertilizers, and nitrification inhibitors in rice grown in different agro-climatic conditions (Carreres *et al*., 2003; Kiran *et al*., 2010), organic matrix entrapped chemical fertilizer (OMECF) used in this study is significantly eco-friendly and low cost due to the use of local, biodegradable, non-toxic, and cost effective agro-waste and can be well affordable for the Indian agrarians.

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