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Organic Matrix Entrapped Bio-fertilizers Increase Growth, Productivity, and Yield of *Triticum aestivum* L. and Transport of NO_3^-, NO_2^-, NH_4^+ and PO_4^{-3} from Soil to Plant Leaves

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ABSTRACT

A consortium of biofertilizers (*Azotobacter chroococcum* and *Bacillus subtilis*) was applied in conventional as well as organic matrix entrapped granular forms as sole nutrient source in two different doses for cultivation of wheat (*Triticum aestivum* L. cv. PBW-343). A double dose of conventional biofertilizers increased the growth of wheat plants as measured on 30, 60, 90, and 120 Days After Sowing (DAS) in terms of root and shoot length, number of roots and leaves, as well as fresh and dry weight of roots and leaves over the recommended dose (0.6 kg ha^{-1}) of the same biofertilizers. The entrapment of biofertilizers in an organic matrix further increased the efficacy of these biofertilizers over the non-entrapped conventional forms. An increase in the plant growth of wheat by application of higher dose of biofertilizers and entrapped biofertilizers was correlated to the availability of NO_3^-, NO_2^- and NH_4^+ in the plant's rhizosphere (0-15 cm) and its transport from soil to the plant leaves as well as productivity and yield of wheat in these experimental fields. The increase of 63.47 and 32.17% in wheat yield was recorded in 120-days old plants by the application of organic matrix entrapped biofertilizers in double dose over no fertilizers and un-entrapped biofertilizers in single dose. The results indicate that efficacy of biofertilizers can be enhanced by increasing the dose of biofertilizers and by providing suitable carriers to replace chemical fertilizers load for wheat cultivation with eco-friendly and organic nutrient technologies.

Keywords: *Azotobacter chroococcum*, *Bacillus subtilis*, Entrapped biofertilizers, Slow release fertilizers, *Triticum aestivum* L.

INTRODUCTION

Wheat (*Triticum aestivum* L., Family-Poaceae) is a major cereal of India, which is the second largest producer of wheat in the world with annual production hovering around 70-75 million tons in the past few years (Joshi *et al*., 2007). The growth, productivity, and yield of wheat largely depend on the type and quantity of fertilizers applied (Gopinath *et al*., 2008). Fertilizers are essential component of agricultural productivity as they provide essential plant nutrients, however, use of synthetic chemical fertilizers are no more considered as ecologically suitable and alternative nutrient sources e.g. organic fertilizers and plant growth promoting rhizobacteria (PGPRs) have been applied to reduce the load of chemical fertilizers (Shekoofa and Emam, 2008; Adesemoye *et al*., 2009; Sharma *et al*., 2011). It has been realized that the excessive use of inorganic fertilizers is unsustainable for any farming practice from economic as well as ecological points of view (Singh *et al*., 2006; 2008a; 2010). Agricultural activities contribute a large percentage of greenhouse gaseous emissions in the form of CH_4, CO_2, N_2O etc. (Akiyama, 2000; Jiang *et al*., 2010). Nitrogen deficiency

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is one of the major yield limiting factors for cereals, hence application of N fertilizers are considered as an essential input to maintain high yield of wheat (Bakht *et al.*, 2009). A major share of the applied inorganic/soluble N is lost through nitrate leaching, surface runoff, volatilization, or emission of N-gases (Adesemoye *et al.*, 2009; Rawat *et al.*, 2010; Weligama *et al*., 2010). Due to decrease in organic matter and micronutrients in intensive cultivation areas, a decline or stagnation in the productivity of wheat has been documented, which persuades farmers for further loading of nitrogenous chemical fertilizers (Heitkamp *et al.*, 2011). Biofertilizers have been identified as an alternative to chemical fertilizers to increase soil fertility and crop production in sustainable farming. These are the products containing living cells of different types of microorganisms, which have the ability to convert nutritionally important elements from unavailable to available forms through biological processes (Wu *et al*., 2005; Kundu *et al.*, 2009). In recent years, biofertilizers have emerged as an important component of the integrated nutrient supply system and hold a great promise to improve crop yield through environmentally better nutrient supplies (Wu *et al*., 2005; Shaukat *et al*., 2006). Strains of *Azotobacter*, *Rhizobium*, *Bradyrhizobium*, *Azospirillum*, *Pseudomonas*, *Bacillus* and *Acetobacter* have been developed as biofertilizers for cereals including wheat, pulses, vegetables, oil seeds, cotton, sugarcane etc. (Mahajan *et al.*, 2003; Ogut *et al.*, 2005; *Shaukat et al.*, 2006; Broschat and Moore, 2007; Adesemoye *et al*., 2009). 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 (Emilsson *et al.*, 2007; Wu *et al.*, 2008; Granta *et al.*, 2012; Kumar *et al*., 2012). These fertilizers play an important role in improving fertilizers use efficiency by plants, thereby mitigating environmental pollution and helping sustainable agriculture (Zhao *et al.*, 2010). No slow release fertilizer has been reported to be applied to wheat (*Triticum aestivum*) fields as per our data base. Although biofertilizer 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.

It has been demonstrated that chemical fertilizers entrapped in organic matrix containing cow dung, clay soil, neem leaves powder and acacia gum (non-toxic and biodegradable organic materials) as a carrier prepared in form of super granules enhances growth, productivity, and yield in rice (Dahiya *et al.*, 2004; Kumar *et al.*, 2012) and Indian Mustard (Sharma and Singh, 2011). No such studies are available for wheat as per our data base. The present study has conducted to assess the effects of enhanced dose of biofertilizers (double of recommended dose) on growth and yield of the plant and availability of inorganic N species (nitrate, nitrite and ammonium) and phosphate in rhizosphere of wheat as well as in plant parts. Moreover, the efforts have made to assess the effects of organic matrix entrapped biofertilizers on these parameters

MATERIALS 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 between 26.30° and 27.10° north latitude and 80.30° and 81.13° east longitude. It has a warm sub-tropical climate with a cool dry winter from December to February. The certified seeds of wheat (*Triticum aestivum* L. cv. PBW- 343) were obtained from a local dealer. The experiments were established in two successive (Rabi) winter seasons of 2009-10 and 2010-11. The experimental design was randomized block plots of five treatments replicated three times. The plot size was 1.5×1 m. The treatments were: (1) no added fertilizer= NF, (2) free (Un-entrapped) form of recommended dose (0.6 kg ha^{-1}) of biofertilizers (*Azotobacter chroococcum* and *Bacillus subtilis* placed in charcoal as carrier) in single dose (UBSD), 3) Free (Un-entrapped) form of the same biofertilizers in double dose (UBDD), 4) Organic matrix entrapped biofertilizers in single dose (EBSD), 5) Organic matrix entrapped biofertilizers in double dose (EBDD).

Entrapment of Biofertilizers in Organic Matrix

Agro-wastes 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 from 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^{-1}) containing a consortium of nitrogen fixing bacteria (*Azotobacter*) and phosphate solubilizing bacteria (*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. Entrapped biofertilizers granules were applied as a basal application in wheat field.

Soil Analysis

Soil samples were collected at the seed sowing and harvesting stages. The top 0-15 cm soil from the vicinity of plant roots was collected and analyzed. Soil pH was measured electrometrically using glass electrode pH meter, model N1G 333 (Jackson, 1967). Organic carbon in the soil samples was estimated by wet digestion method of Walkley and Black (1934). Available nitrogen in soil was estimated by using the alkaline potassium permanganate (Subbiah and Asija, 1956). Available phosphorus in soil was estimated by the method of Olsen *et al.* (1954). Soluble potassium was estimated by the method described by Jackson (1958).

Measurement of Plant Parameters

The root and shoot length were measured in plants at the age of 30, 60, 90, and 120 days after sowing (DAS) using meter scale. The plant parts were 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. One leaf and one root in six replicates for each treatment were oven dried at 70°C, till a constant dry weight was recorded.

Estimation of Nitrate, Nitrite, Ammonium, and Phosphate Content

Nitrate content in soil and leaves were estimated by the method described by Cataldu *et al.* (1975), by using 5% salicylic acid solution in concentrated sulfuric acid and 2N sodium hydroxide. Nitrite content in soil and leaves were estimated by the method described by Steven and Oaks (1973), using homogenate 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 method using 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).

Statistical Analysis

All treatments were replicated three times with two measurements in each experimental plot (n= 6). Results were analyzed using One-way ANOVA (SPSS statistical package and MS excel). The differences between treatments were considered as non-significant (ns), *significant at $P< 0.05$, and **significant at $P< 0.01$.

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RESULTS

Soil Characteristics

The soil pH decreased from 8.11 to 7.67 in UBSD and from 8.07 to 7.25 in EBDD, respectively, at seed sowing (SS) and harvesting (H). In EBDD, there was an increase of 50.01% (SS) to 63.55% (H) in water holding capacity (WHC), from 0.47 (SS) to 0.78% (H) in organic carbon, from 0.81 (SS) to 1.34% (H) in organic matter, from 632 (SS) to 1072 kg ha^{-1} (H) in total N, from 58.21 (SS) to 210.21 kg ha^{-1} (H) in available N, from 8.91 (SS) to 23.26 kg ha^{-1} (H) in available P_2O_5, and from 75.98 (SS) to 262.16 kg ha^{-1} (H) in soluble K (Table 1). Water holding capacity, organic carbon, organic matter, total N, available N, available P_2O_5 and soluble K were also increased in the entrapped organic matrix based biofertilizers compared to the un-entrapped biofertilizers (Table 1).

Growth Parameters

Application of the biofertilizers i.e. consortium of *Azotobacter chroococcum* and *Bacillus subtilis,* increased root length, number of roots, and fresh and dry weight of roots significantly compared to no-fertilizer treatments, on 30, 60, 90, 120 DAS. The increase in the plant growth was, however, more pronounced when measured on 30 DAS (Table 2). An enhanced dose of un-entrapped as well as organic matrix entrapped biofertilizers increased the plant growth significantly. The entrapment of biofertilizers (single and double dose) in the organic matrix prepared under this study enhanced the root length by 23.59 and 29.35%, respectively, over free forms of biofertilizers at 120 DAS (Table 1). At the rate of 1.2 kg ha^{-1}, biofertilizers in entrapped form caused a very significant increased in the root biomass compared to the recommended dose (0.6 kg ha^{-1}) and non-entrapped biofertilizers. The increase in root growth due to the entrapped and enhanced dose of biofertilizers was consistent at all four ages of the plant (30, 60, 90, 120 DAS). The

Table 1. Effect of different doses of un-entrapped and organic matrix entrapped biofertilizers on soil at seed sowing (SS) and harvesting (H) stages.[a]

Soil property		NF	UBSD	UBDD	EBSD	EBDD
pH	SS	8.01±0.31	8.11±0.41	8.08±0.48	8.02±0.38	8.07±0.33
	H	7.88±0.36	7.67±0.39	7.60±0.42	7.33 ±0.51	7.25±0.48
WHC %	SS	50.22±4.01	49.11±3.98	49.55±3.05	50.00±4.45	50.01±5.01
	H	58.01±5.11	60.22±4.75	62.21±5.86	63.01±6.01	63.55±5.22
Organic Carbon%	SS	0.48±0.04	0.47±0.03	0.48±0.03	0.48±0.05	0.47±0.04
	H	0.60±0.03	0.70±0.04	0.72±0.05	0.76±0.05	0.78±0.04
Organic Matter%	SS	0.83±0.06	0.81±0.07	0.83±0.07	0.83±0.06	0.81±0.07
	H	1.03±0.09	1.20±0.11	1.24±0.08	1.31±0.12	1.34±0.13
Total N (kg ha^{-1})	SS	630±21.02	650±22.33	645±22.45	635±20.67	632±20.88
	H	950±35.45	1050±37.65	1060±30.88	1061±38.98	1072±37.88
Available N (kg ha^{-1})	SS	55.03±3.22	59.04±4.02	53.33±3.88	57.21±4.56	58.21±3.01
	H	180.11±8.98	220.34±10.43	240.18±12.32	200.16±11.21	210.20±12.32
Available P_2O_5 (kg ha^{-1})	SS	9.01±0.86	9.32±0.78	10.21±0.91	9.00±0.79	8.91±0.81
	H	15.10±1.23	18.19±1.98	18.25±1.01	20.15±1.38	23.26±1.58
NH_40Ac Soluble K (kg ha^{-1})	SS	75.02±5.03	78.33±5.01	76.43±5.21	74.87±6.01	75.98±5.22
	H	150.16±10.02	180.23±12.21	200.16±13.56	235.55±16.09	262.16±17.82

[a] All the values are means of three replicates with two determinations (n= 6)±SD. Where, NF: No Fertilizers; UBSD: Un-entrapped Biofertilizers in Single Dose; UBDD: Un-entrapped Biofertilizers in Double Dose; EBSD: Organic matrix Entrapped Biofertilizers in Single Dose, EBDD: Organic matrix Entrapped Biofertilizers in Double Dose.

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Table 2. Effect of different doses of un-entrapped and organic matrix entrapped biofertilizers on growth of wheat (*Triticum aestivum* L. cv. PBW-343) roots on 30, 60, 90 and 120 days after sowing (DAS).[a]

Parameter	Treatment	30 d	60 d	90 d	120 d
Root Length ($cm\ plant^{-1}$)	NF	1.47±0.31	3.50±0.40	5.50±0.50	5.73±0.40
	UBSD	4.23± 0.31*	4.47±0.25 ns	6.70±0.44*	7.63±1.10**
	UBDD	4.97.0±0.78*	5.87±0.97**	7.50±0.87**	8.87±0.76**
	EBSD	5.37±0.70*	7.30±0.26**	8.57±0.60**	9.43±0.55**
	EBDD	5.77±0.42*	7.57 ±1.44**	8.80±0.36**	9.87±0.25**
Number of roots (piece $plant^{-1}$)	NF	2.67±0.58	8.33±1.15	9.33±0.58	11.33±0.58
	UBSD	4.67±0.58 ns	8.67±0.58 ns	16.00±2.00**	16.00±2.00**
	UBDD	5.67±1.15*	12.67±1.53**	19.67±1.53**	22.67±0.58**
	EBSD	7.33±1.15**	17.00±1.00**	21.67±1.15**	24.00±2.00**
	EBDD	9.00±2.00**	19.00±1.00**	23.00±1.00**	26.00±1.00**
Fresh wt. of roots ($g\ plant^{-1}$)	NF	0.12±0.03	0.13±0.02	0.20±0.02	0.24±0.03
	UBSD	0.20±0.02*	0.23±0.02**	0.35±0.06**	0.38±0.11*
	UBDD	0.22±0.03**	0.27±0.03**	0.36±0.03**	0.50±0.03**
	EBSD	0.38±0.05**	0.55±0.05**	0.59±0.04**	0.67±0.02**
	EBDD	0.44±0.05**	0.58±0.03**	0.67±0.03**	0.73±0.04**
Dry wt. of roots ($g\ plant^{-1}$)	NF	0.02±0.01	0.07±0.01	0.07±0.02	0.08±0.01
	UBSD	0.07±0.03**	0.09±0.02 ns	0.11±0.01**	0.12±0.01**
	UBDD	0.10±0.01**	0.09±0.01 ns	0.12±0.01**	0.15±0.02**
	EBSD	0.11±0.01**	0.13±0.02**	0.12±0.01**	0.13±0.01**
	EBDD	0.12±0.01**	0.13±0.02**	0.15±0.02**	0.14±0.02**

[a] All the values are means of three replicates with two determinations (n=6)±SD, (one way ANOVA). ns= Not significant; *= $P< 0.05$, **= $P< 0.01$. Treatment symbols are defined in the text and under Table 1.

increase of 76.32 and 92.11% in fresh weight and 75 and 62% in dry weight of root were recorded in 120-day old plants by the application of, respectively, EBSD and EBDD over free form of biofertilizers in single dose (Table 2).

Application of the single and double dose of the recommended rate of the biofertilizers (consortium of *Azotobacter chroococcum* and *Bacillus subtilis*) increased shoot length, no. of leaves, and fresh and dry weight of shoots significantly compared to no fertilizers application (Table 2). The entrapment of biofertilizers in the organic matrix prepared under this study enhanced the shoot length by 8.07 and 12.94%, respectively, over free forms of biofertilizers at 120 DAS (Table 2). At the rate of 1.2 kg ha^{-1}, biofertilizers in entrapped from caused a very significant increase in the shoot biomass relative to the recommended dose (0.6 kg ha^{-1}) and non-entrapped biofertilizers. The increase in shoot length due to the entrapped and enhanced dose of biofertilizers was consistent at all four ages of the plants (30, 60, 90, 120 DAS). The percentage increase of 155 and 225.45% in fresh weight and 141.20 and 153.76% in dry weight of shoot were recorded in 120-days old plants by the application of, respectively, EBSD and EBDD over free form of biofertilizers in single dose (Table 3).

Tiller number was also affected significantly by the application of free biofertilizers and organic based entrapped biofertilizers (Figure 1-A). The increase of 77.67% in tiller number was recorded in 90-days old plants by the application of EBDD over free biofertilizers. On 120 DAS tiller number was increased by 225.56% due to the application of EBDD over the no fertilizer and 85.84% over the recommended dose of un-entrapped biofertilizers.

The enhanced and entrapped biofertilizers increased seed yield significantly over NF or single dose (recommended dose) of biofertilizers (Figure 2). The increase of 63.47 and 32.17% in wheat yield was recorded in 120-days old plants by the application of

Table 3. Effect of different doses of un-entrapped and organic matrix entrapped biofertilizers on growth of wheat (*Triticum aestivum* L. cv. PBW-343) shoots on 30, 60, 90 and 120 days after sowing (DAS).[a]

Parameters	Treatment	30 d	60 d	90 d	120 d
Shoot length (cm)	NF	13.27±1.80	24.23±2.50	42.10±2.57	46.20±3.50
	UBSD	15.37±5.15 ns	35.77±2.71**	51.10±5.09*	54.87±0.92**
	UBDD	16.80±2.84 ns	37.03±0.51**	53.50±1.44**	56.80±0.79**
	EBSD	18.29±1.69 ns	38.23±1.33**	55.33±4.58**	59.30±0.75**
	EBDD	19.00±0.56*	46.13±1.97**	58.60±2.58**	61.97±2.04**
Number of leaves	NF	3.00±1.00	5.33±1.15	7.33±1.15	7.00±1.00
	UBSD	5.67±0.58**	9.00±1.00**	10.67±1.53**	9.67±1.15**
	UBDD	6.00±1.00**	12.33±0.58**	13.33±0.58**	11.33±0.58**
	EBSD	6.33±1.15**	14.67±1.53**	15.00±1.00**	14.00±1.00**
	EBDD	7.00±1.0**	16.00±1.00**	17.00±1.00**	15.67±0.58**
Fresh wt of shoot (g)	NF	0.43±0.11	0.69±0.04	1.30±0.50	3.08±0.17
	UBSD	1.08±0.13 ns	2.61±0.01**	3.07±0.13**	4.40±0.50**
	UBDD	1.79±0.57**	3.12±0.10**	3.52±0.10**	6.25±0.21**
	EBSD	2.69±0.29**	3.06±0.14**	5.25±0.68**	11.25±0.39**
	EBDD	3.86±0.61**	4.07±0.05**	7.70±0.56**	14.32±0.37**
Dry wt. of shoots (g)	NF	0.12±0.03	0.14±0.03	0.36±0.05	0.69±0.04
	UBSD	0.39±0.06**	0.58±0.04**	0.91±0.15**	1.99±0.03**
	UBDD	0.60±0.02**	0.71±0.06**	1.04±0.06**	2.28±0.31**
	EBSD	0.92±0.03**	0.92±0.06**	1.51±0.34**	4.80±0.23**
	EBDD	1.23±0.18**	1.01±0.03**	2.19±0.23**	5.05±0.48**

[a] All the values are means of three replicates with two determinations (n=6)±SD, (one way ANOVA). ns= Not significant; *= $P< 0.05$, **= $P< 0.01$. Treatment symbols are defined in the text and under Table1.

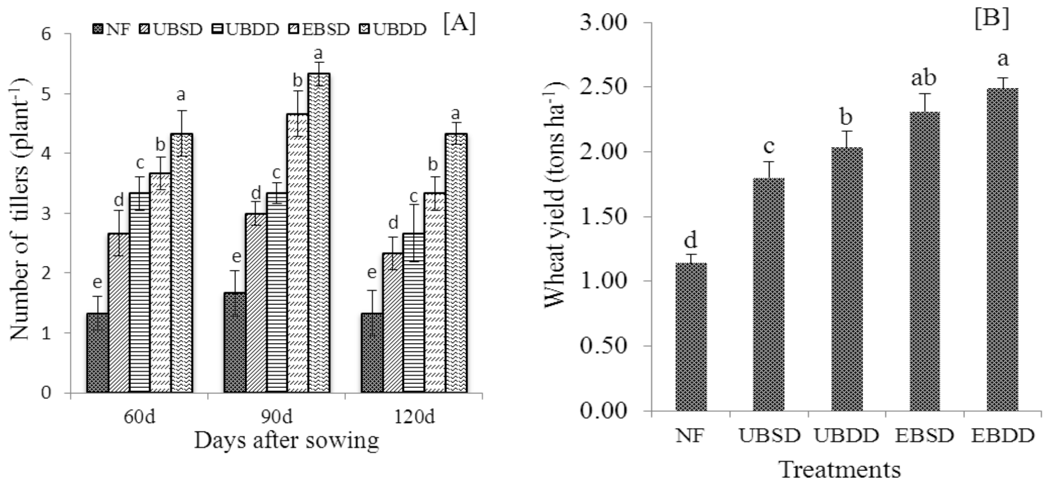

Figure 1. Effect of entrapped and un-entrapped biofertilizers on tiller numbers at 60, 90 and 120 DAS (A) and wheat yield at 120 DAS (B). All the values are means of three replicates with two determinations (n= 6)±SD (one way ANOVA). Values followed by different letters show significant differences between the treatments at $p<0.05$. Treatment symbols are defined in the text and under Table 1.

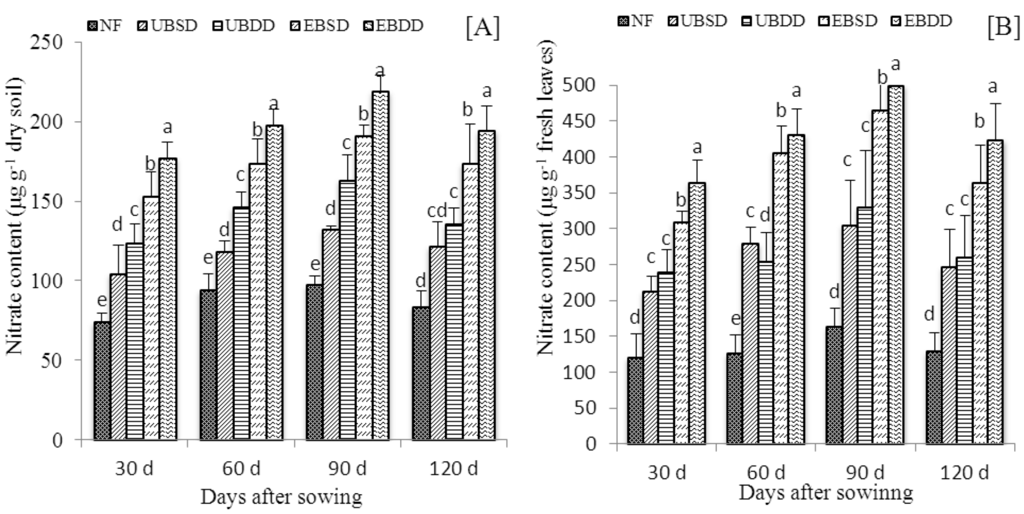

Figure 2. Levels of Nitrate (µg g^{-1}) in dry soil [A] and leaves [B] of *Triticum aestivum* L. at 30, 60, 90 and 120 DAS in different treatments Treatment symbols are define in the text and under Table 1. Values followed by different letters show significant differences between the treatments at *P*< 0.05.

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EBDD over, respectively, NF and UBSD (Figure 1-B).

Level of Nitrate, Nitrite, and Ammonium Content in Rhizosphere and Leaves

The soil nitrate increased by 139.44, 111.11, 124.99, and 133.33%, respectively, on 30, 60, 90 and 120 DAS with application of the entrapped biofertilizers (double dose) over that in the control (non-fertilized), 70, 67.64, 65.79 and 60% over the recommended dose of un-entrapped fertilizers, and 43.39, 35.71, 34.04 and 43.58% over the single dose of un-entrapped biofertilizers (Figure 2-A).

Nitrate, nitrite, and ammonium contents in fresh leaves of wheat plants were increased in treatments of entrapped organic matrix based biofertilizers in double dose compared to no fertilizer and free form of biofertilizers treatments. Single dose of free biofertilizers applied plants had lower nitrate content in leaves of the plant in comparison to higher dose and organic matrix entrapped biofertilizers applied plant leaves (Figure 2-B).

Nitrate content in wheat leaves applied with enhanced dose (1.2 kg ha^{-1}) of organic matrix entrapped biofertilizers increased by 204.28% and 72.13% on 30 DAS, 241.99 and 54.42% on 60 DAS, 206.39 and 63.82% on 90 DAS, and by 229.72 and 71.83% on 120 DAS over, respectively, the no-fertilizer and the recommended dose (0.6 kg ha^{-1}) of un-entrapped biofertilizers (Figure 2-A). The organic matrix entrapped biofertilizers with enhanced dose also increased soil nitrate content of the plant's rhizosphere at the depth of 0-15 cm (Figure 2-B). Correlation between average soil nitrate and average plant leaves nitrate at 120 DAS in different treatments was linearly significant (R^2= 0.968) (Figure 3).

The soil nitrite increased by 167.74, 121.05, 97.78 and 108.38%, respectively, on 30, 60, 90, and 120 DAS with application of the entrapped biofertilizers (double dose) over that in the control (no fertilizers). The corresponding increase was 27.69, 23.53, 25.35, and 37.09% over the recommended dose of un-entrapped fertilizers, and 22.06, 18.31, 23.61, and 26.87% over the same amount of un-entrapped biofertilizers (Figure 4-B).

Nitrite content in wheat leaves applied with enhanced dose (1.2 kg ha^{-1}) of organic matrix entrapped biofertilizers increased by 222.84 and 66.93% on 30 DAS, 240.09 and 65.54%

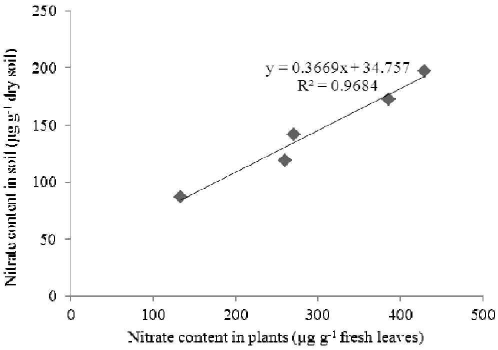

Figure 3. Correlation between average soil nitrate and average plant leaves nitrate (120 DAS) in different treatments.

on 60 DAS, 227.39 and 64.83% on 90 DAS, 261.90 and 71.00% on 120 DAS over, respectively, the no-fertilizer and the recommended dose (0.6 kg ha^{-1}) of un-entrapped biofertilizers (Figure 4-A). The organic matrix entrapped biofertilizers with enhanced dose also increased soil nitrite content of the plant's rhizosphere at the depth of 0-15 cm (Figure 4-B). Correlation between average soil nitrite and average plant nitrite at 120 DAS in different treatments was linearly significant (R^2= 0.873) (Figure 5).

The soil ammonium increased by 253.79, 262.16, 250.93, and 295.62%, on, respectively, 30, 60, 90 and 120 DAS with application of the entrapped biofertilizers (double dose) over that in the control. The corresponding increase was 52.68, 52.27, 51.07, and 46.09% over the recommended dose of un-entrapped fertilizers and 38.27, 34, 31.70, and 44.91% over the same amount of un-entrapped biofertilizers (Figure 6-A).

Ammonium content in wheat leaves applied with enhanced dose (1.2 kg ha^{-1}) of organic matrix entrapped biofertilizers increased by 253.79 and 52.68% on 30 DAS, 262.16 and 52.27% on 60 DAS, 250.93 and 51.07% on 90 DAS, and 295.62 and 46.09% on 120 DAS over, respectively, the control and the recommended dose (0.6 kg ha^{-1}) of un-entrapped biofertilizers (Figure 6-B). The organic matrix entrapped biofertilizers with enhanced dose also increased soil ammonium content of the plant's rhizosphere at the depth of 0-15 cm (Figure 6-A). Correlation between average soil ammonium and average plant ammonium at 120 DAS in different treatments was linearly significant (R^2= 0.956) (Figure 7).

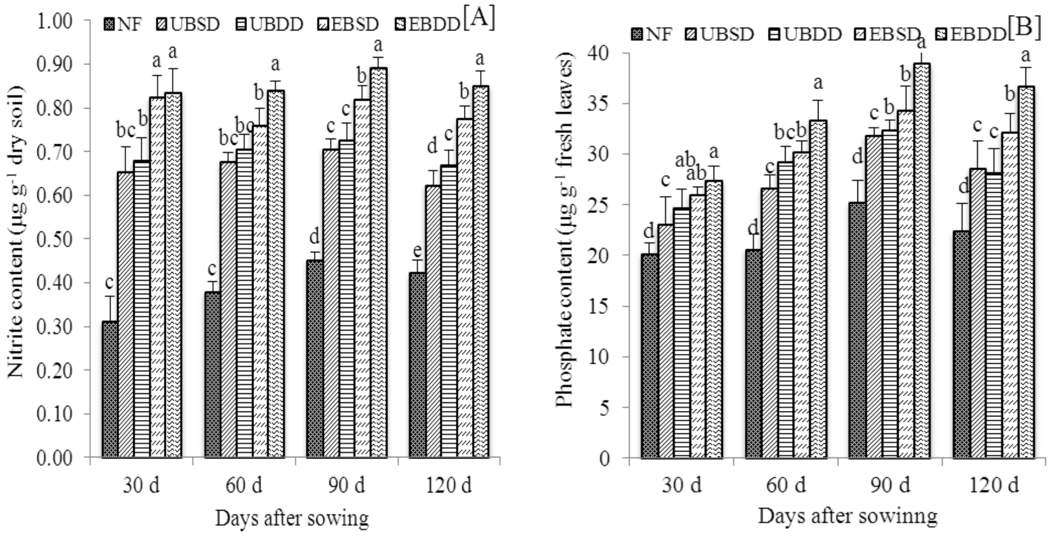

Figure 4. Levels of phosphate (µg g^{-1}) in dry soil [A] and leaves [B] of *Triticum aestivum* L. at 30, 60, 90 and 120 DAS in different treatments (other details are described in Figure 1). Values followed by different letters are significantly differences between the treatments at P< 0.05.

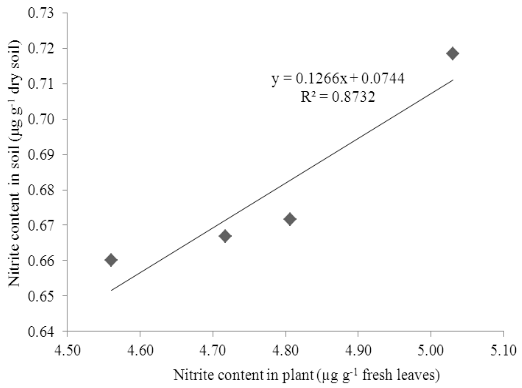

Figure 5. Correlation between average soil nitrite and average plant leaves nitrite (120 DAS) in different treatments.

Phosphate Content in Rhizosphere and Leaves

The soil phosphate increased by 307.45, 294.17, 291.67, and 325.00% on, respectively, 30, 60, 90, and 120 DAS with application of the entrapped biofertilizers (double dose) over that in the control, 78.14, 76.52, 69.88, and 74.35% over the recommended dose of un-entrapped fertilizers, and 38.76, 36.70, 37.33, and 44.17% over the same amount of un-entrapped biofertilizers (Figure 8-A).

Phosphate content in fresh leaves of wheat plants were increased in treatment of organic matrix entrapped biofertilizers in double dose in comparison to the control and the free form of biofertilizers. Single split dose of free biofertilizers-applied plants had lower phosphate component in comparison to higher dose and also the organic matrix entrapped biofertilizers applied plant leaves (Figure 8-B).

Phosphate content in wheat leaves applied with enhanced dose (1.2 kg ha^{-1}) of organic matrix entrapped biofertilizers increased by 35.88 and 19.14% on 30 DAS, 56.43 and 24.99% on 60 DAS, 54.51 and 22.22% on 90 DAS, and 63.88 and 22.34% on 120 DAS over, respectively, the control and the recommended dose (0.6 kg ha^{-1}) of un-entrapped biofertilizers (Figure 8-B). The organic matrix entrapped biofertilizers with enhanced dose also increased soil ammonium content of the plant's rhizosphere at the depth of 0-15 cm (Figure 8-A).Correlation between average soil phosphate and average plant leaves phosphate at 120 days in different treatment was linearly significant (R^2= 0.969) (Figure 9).

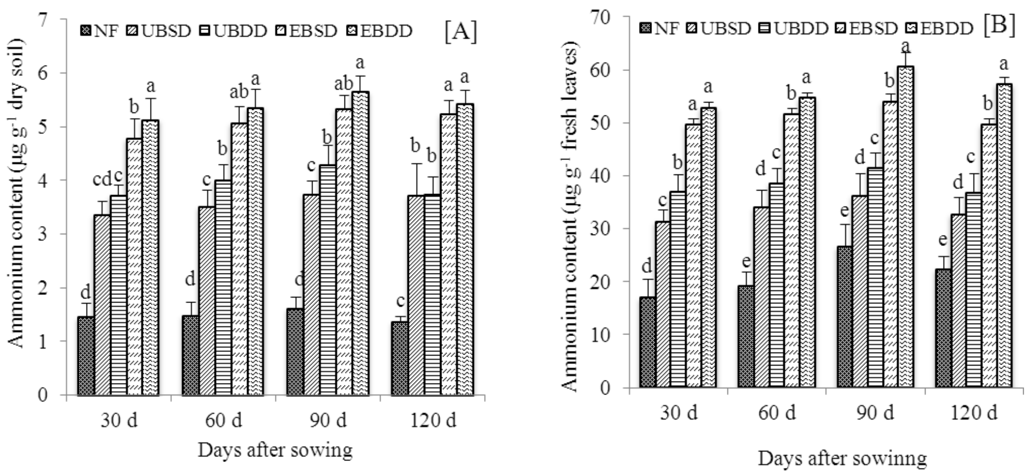

Figure 6. Levels of ammonium ion (µg g^{-1}) in dry soil [A] and fresh leaves [B] of *Triticum aestivum* L. at 30, 60, 90 and 120 DAS in different treatments (other details are described in Figure 1). Values followed by different letters are significantly differences between the treatments at *P*< 0.05.

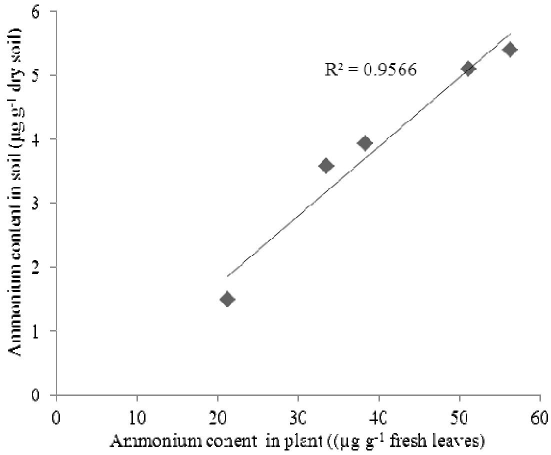

Figure 7. Correlation between average soil ammonium and average plant ammonium (120 DAS) in different treatments.

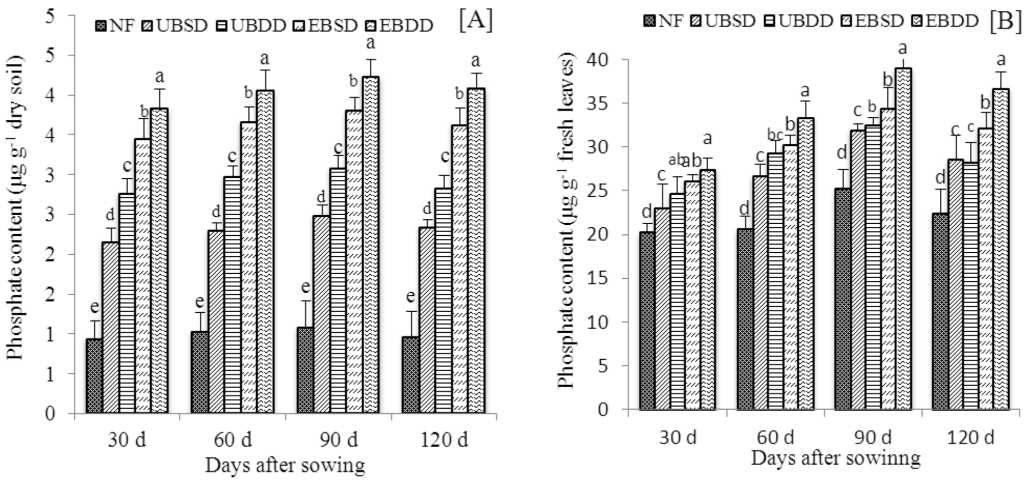

Figure 8. Levels of phosphate (µg g^{-1}) in soil [A] of *Triticum aestivum* L. and fresh leaves [B] at 30, 60, 90 and 120 DAS in different treatments (as defined in Figure 1). Values followed by different letters are significantly differences between the treatments at *P*< 0.05.

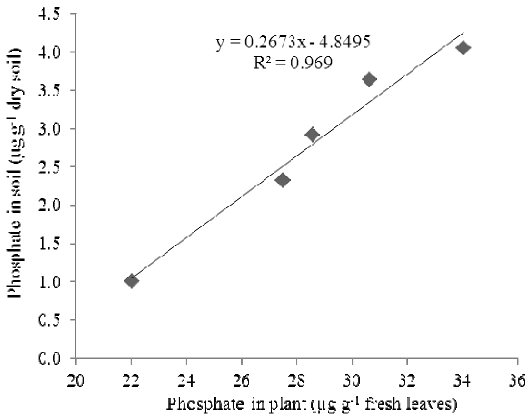

Figure 9. Correlation between average soil phosphate and average plant leaves phosphate (120 DAS) in different treatments.

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DISCUSSION

The N cycle is an essential and complex biogeochemical cycle that has a great impact on soil fertility (Miranzadeh *et al*., 2011; Jetten, 2008). The low nutrient levels in soil lead to low crop productivity due to less availability of essential nutrients needed for plant growth, metabolism and reproductive yield. Therefore, additional fertilizers (especially N fertilizers) are applied to increase crop yield. Since application of fertilizers is directly related to plant yield in cereals like wheat, excessive loading is a common feature in green revolution belts, which causes many environmental, economic, and health related problems (Singh *et al*., 2008a, b and 2010; Abedi *et al*., 2010; Cerny *et al*., 2010). Low efficiency of the uptake of fertilizers in many crops is another factor that aggravates the leaching, volatilization, and emissions related losses of the added soluble chemical fertilizers, which are readily released in the soil and atmosphere (Akiyama, 2000). Over 50% of the applied N can be lost from agricultural systems as N trace gases and reactive nitrogen species (Adesemoye *et al*., 2009; Weligama *et al*., 2010). Similarly when P is applied in high percentage in comparison to other nutrients, sometimes up to 90%, is precipitated by metal complexes in the soil and can later lead to P pollution (Adesemoye *et al*., 2009).

Nitrate leaching and runoff in agricultural fields have been well documented and can lead to eutrophication and death of aquatic life due to O_2 deficiency (Diez *et al*., 1997; Weligama *et al*., 2010). The organic fertilizers and various kinds of customized fertilizers e.g., slow release fertilizers, controlled released fertilizers, urease and nitrification inhibitors as well as microbial biofertilizers have a better retention and continuity of release of the nutrients in plants rhizosphere, therefore, their application can reduce the environmental losses of the expensive plants nutrients during the crop cultivation (Dahiya *et al*., 2004; Sieling *et al*., 2006; Emilsson, 2007; Zamen and Blennerhassett, 2010). A direct correlation between the application of NPK fertilizers and crop productivity have been reported for wheat cultivation (Yadav, 2003; Kumar and Nanwal, 2006; Osborne, 2007), which may lead to leaching, vitalization and emission losses (Akiyama *et al*., 2000; Weber *et al*., 2001; Wei-xin *et al*., 2007; Jiang *et al*., 2010). Biofertilizers, e.g. *Azotobacter chroococcum*, *Bacillus subtilis, Azospirillum*, *Acetobacter* (Kumar *et al*., 2001; Ogut *et al*., 2005) and organic fertilizers (Sharma and Prasad, 1999) have been applied to wheat fields as alternative and eco-friendly nutrients. The integrated nutrient management practices and use of customized fertilizers have also been attempted (Kumar and Nanwal, 2006; Sharma *et al*., 2011). The data presented in this paper indicate that the application of a biofertilizer consortium in double dose of the recommended dose and its entrapment in an organic matrix, earlier used by our group to entrap chemical fertilizers like urea and ammonium sulphate (Dahiya *et al*., 2004; Sharma and Singh, 2011), increase the availability of nitrate, nitrite, ammonium, and phosphate in wheat rhizosphere and in plant leaves, which are directly correlated to the growth and productivity of the plants (Tables 1-2, Figures 1-9).

The results indicate that the dose of biofertilizers usually used for wheat is not a true reflection of the actual requirements of biofertilizers for different crops in different agro-climatic regions and it requires a revisit. In our case, double dose of biofertilizers provide better nutrient availability and crop productivity. In this case, we have not optimized the optimal dose of this biofertilizer, which is planned for future. In addition, entrapment of these biofertilizers to a biodegradable and low cost organic matrix that contained local and cheap agro-waste materials like cow-dung, neem leaf powder, clay soil, and Acacia gum saresh enhanced its efficacy over the free form of biofertilizers. This opens a new dimension to develop commercial organic fertilizers that can maintain the crop productivity parallel to the conventional chemical fertilizers and simultaneously can be eco-friendly, cost effective, and soil enriching.

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کود زیستی با پوشش ماده آلی، رشد ، بهره وری و عملکرد گندم *Triticum aestivum* L. و انتقال $NH4^+$, $NO2^-$, $NO3^-$و$PO4^{-3}$ را از خاک به برگ افزایش میدهد

س. کومار، ک. بوده، س. س. بارمن، ر. پ. سینگ

چکیده

مجموعه ای از کود های زیستی (*Azotobacter chroococcum*و *Bacillus subtilis*) در شکل رایج آن ونیز به صورت دانه ای با پوشش مواد آلی به عنوان تنها منبع عناصر غذایی در دو مقدار مختلف در کشت گندم (*Triticum aestivum* L. cv. PBW-343)به خاک داده شد.اندازه گیری رشد گندم درروزهای ۱۲۰،۹۰،۶۰،۳۰ روز پس از بذرکاری نشان داد که افزایش کود زیستی با روش رایج به دو برابر مقدار توصیه شده(۰/۶ کیلو گرم در هکتار) باعث شد که طول ریشه و ساقه، تعداد ریشه وبرگ و نیز وزن تر وخشک ریشه وبرگ ها در مقایسه با مقدار توصیه شده زیادتر شود. پوشاندن کود زیستی مزبور با ماده های آلی ، کار آمدی این کود ها را در مقایسه با کودهای زیستی رایج بدون پوشش افزایش داد. نتایج حاکی از آن بود که افزایش های مزبور با مقدار فراهمی (در دسترس بودن)NO^{3-} ، NO^{2-}، و NH^{4+} در ریشه گاه (۰–15cm) و نیز با انتقال این یون ها از خاک به برگ گیاهان و همچنین با بهره وری و عملکرد گندم در مزارع آزمایشی همبستگی داشت.اندازه گیری های ثبت شده ۱۲۰ روز بعد از بذر کاری نشان داد که درتیمار کود زیستی پوشش دار به میزان دو برابر مقدار توصیه شده، افزایش عملکرد گندم در مقایسه با تیمار بدون مصرف کود و تیمار مصرف کود زیستی به مقدار توصیه شده به ترتیب برابر ۶۳/۴۷٪ و۳۲/۱۷٪ بود. نتایج به دست آمده چنین اشاره دارد که می توان کار آمدی کودهای زیستی را با افزایش مقدار مصرف آن و تهیه حامل های مناسب بالا برد و آن ها را به عنوان ماده های دوست–زیست بوم و فرآورده های فناوری عناصر غذایی آلی به جای کود های شیمیایی در کشت گندم به کار برد.

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