Int.J.Curr.Microbiol.App.Sci (2018) Special Issue-7: 3221-3238

EXCELLENT PUBLISHERS

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Special Issue-7 pp. 3221-3238**
Journal homepage: http://www.ijcmas.com

Original Research Article

Studies on Growth, Mobilization of Nutrients and Yield of Wheat (*Triticum aestivum* L. PBW - 343) Applied with Organic Matrix Based Slow Release Bio Fertilizers

Indra Jeet Chaudhary* and Rana Pratap Singh

Department of Environmental Sciences, Babasaheb Bhimrao Ambedkar University
(A central University) Lucknow -226025, India
**Corresponding author*

Keywords

Organic matrix, Azotobactor, PSB, EBFA, EBFM

A B S T R A C T

Plant nutrients are essential for the growth productivity of crops and food for the world's increasing population. Plant nutrients are a major component of sustainable agriculture. A consortium of bio fertilizers (Azotobacter chroococcum and Bacillus subtilis, PSB) was applied in free form as well as organic matrix entrapped granular forms (Cow dung, Neem leaves powder, clay soil) in single and double doses of different mode of application for cultivation of wheat (*Triticum aestivum* L. cv. PBW-343). A double dose of conventional bio fertilizers enhanced the growth & productivity of wheat plants measured on 20, 40, 60, and 120 Days after sowing (DAS).The entrapped of bio fertilizers in an organic matrix further increased the efficacy of these bio fertilizers over the non-entrapped conventional forms. An increase in the plant growth of wheat by application of entrapped bio fertilizers was to maintain availability of nitrate, nitrite and ammonium in the rhizosphere zone of plant and transport it to the plant leaves. The treatment was applied twice on 20 and 40 DAS, increased (EBFA, 48.71%) and (EBFM, 36.66%) over no fertilizer plant of yield. The results indicate that efficacy of bio fertilizers can be enhanced by increasing the dose of bio fertilizers and by providing suitable carriers to replace chemical fertilizers load for wheat cultivation with eco-friendly and organic nutrient technologies.

Introduction

Wheat (*Triticum aestivum* L., Family-Poaceae) is a major cereal crop of India and second largest producer in the world with annual production growing around 70-75 million tons in the past few years (12). The growth, productivity, and yield of wheat largely depend on the type and quantity of fertilizers application (8). Fertilizers are essential for agricultural productivity and provide necessary 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 (21, 1, 18). It has been comprehended that the excessive use of inorganic fertilizers is unsustainable for any farming practices from economic as well as ecological points of view (22, 23, 24). Agricultural activities contribute a large percentage of greenhouse gaseous emissions in the form of CH_4, CO_2, N_2O etc. (2, 11). Nitrogen deficiency is one of the major yield limiting factors for cereals, hence application of nitrogen fertilizers are considered as an essential input to maintain high yield of wheat (3). A major part of the applied inorganic/soluble nitrogen is lost through nitrate leaching, surface runoff, volatilization and emission of nitrogen-gases (1, 17, 27). Due to decrease inorganic matter and micronutrients in intensive cultivation areas, a decline or inaction in the productivity of wheat has been documented, which encourages farmers for further loading of nitrogenous chemical fertilizers (10). Bio fertilizers have been recognized as an alternative to chemical fertilizers to increase soil fertility and crop production in sustainable farming. These bio fertilizers containing living cells of different types of microorganisms which have the ability to conversion of nutritionally important elements from unavailable to available forms through biological processes (29, 14). In recent years, bio fertilizers have emerged as an important component of the combined nutrient supply system and yield through environmentally better nutrient supplies which carries great promises to improve crop yield (29, 20).

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. (15, 16, 20, 4, 1). Slow release bio fertilizers are also produced by the technical intermediations which breakdown the nutrients and make them available to the plants for longer duration (7, 28, 9, 13). These fertilizers play an important role in improving growth and development of plants, there by mitigating environmental pollution and helping in sustainable agriculture (30). Although bio fertilizer offers an economically 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 of 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 of rice (6, 13) and Indian Mustard (19). The present study was conducted to assess the effects of bio fertilizers with different mode of applications (single and double doses) on growth and yield of the plant. Whereas it also make them available in the form of inorganic nitrogen species (nitrate, nitrite and ammonium) and phosphate in rhizospheric zone of wheat as well as in plant parts. Moreover, the efforts have made to assess the effects of organic matrix entrapped bio fertilizers on the basis of growth productivity and biochemical changes.

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 º and 27.10º north latitude and 80.30 º and 81.13 º east longitude. It has a warm sub-tropical climate with a cool dry winter from November to February. The certified seeds of wheat (*Triticum aestivum* L.cv. PBW- 343) were obtained from a local dealer. The experiments were established in (Rabi) winter seasons of 2012. The experimental design was randomized in pots of 14 treatments replicated three times. The pots size was 14×24 cm. The treatments were: described in (table 1).

Entrapment of Bio fertilizers in Organic Matrix

Agro waste like cow dung, Neem leaves (*Azadirachta Indica*) and clay soil (diameter of particles < 0.002 mm) was collected locally. All the collected material were dried separately in an oven at 60 degree centigrade for 3-days and powdered in a grinder. This supporting matrix was then mixed in ratio 1:1:1 ratios, **(**15 gm. cow dung + 15 gm. neem leaves powders + 15 gm. clay soils +0.54 gm. *Azotobacter* and PSB (phosphate solubilizing bacteria)) and then bind with Saresh (plant gum) and molasses. Granule of slow release bio fertilizer (SRF_S) were prepared manually dried in oven and packed in polythene bags.

Measurement of Plant growth & productivity

The root and shoot length were measured in plants at the age of 20, 40 and 60 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 three replicates for each treatment was oven dried at 80°C, till a constant dry weight was recorded. Yield of plant was measured by seed weight (gm/plant).

Estimation of Nitrate, Nitrite, Ammonium, and Phosphate Content

Nitrate content in soil, root and leaves were estimated by the method described by Cataldu *et al.,* (5), by using 5% salicylic acid solution in concentrated sulfuric acid and 2N sodium hydroxide. Nitrite content in soil, root and leaves were estimated by the method described by Steven and Oaks (25), using homogenate of the sample with sulphanilamide and N- (1-Naphthyl)-ethylene-diamine dihydrochloride. Ammonium content in soil, root and leaves were estimated by the method described by Weather burn (26), 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, carry100 Bio).

Statistical analysis

Data were analysed using Deccan's tested through SPSS software (SPSS Inc., version 10.0) to assess the level of significance of quantitative changes due to slow release bio fertilizer treated plant in different parameters and different samplings.

Results and Discussion

Soil Characteristics

The soil pH decreased from 8.3 to 7.57 in EBFA and from 8.2 to 7.1 in EBFM, respectively, at seed sowing (SS) and harvesting (H). In EBFM, there was an increase of 51.01% (SS) to 62.53% (H) in water holding capacity (WHC), from 0.57 (SS) to 0.87% (H) in organic carbon, from 0.82 (SS) to 1.24% (H) in organic matter, from 642 (SS) to 1172 kg ha^{-1} (H) in total nitrogen, from 57.22 (SS) to 210.21 kg ha-1 (H) in available nitrogen and from 9.1 (SS) to 23.26 kg ha-1 (H) in available P_2O_5.Water holding capacity, organic carbon, organic matter, total N, available N and available

P_2O_5 were also increased in the entrapped organic matrix based bio fertilizers compared to the un entrapped bio fertilizers.

Growth parameter

Application of fertilizers applied in wheat soil at 0, 20, Twice applied (20 and 40 DAS) and 40 days after sowing. Application of the treatment twice applied in 20 and 40 DAS increased root length, shoot length, no. of roots, no. of leaves, fresh wt. and dry wt. of roots and shoots over the other mode of applications (Table 2-4).

Root and shoot length

Organic matrix entrapped Bio fertilizers bind with acacia (EBFA) and molasses (EBFM) increased root length 58.50 and 57.45% in 20 DAS, 32.41 and 29.65% in 40 DAS, 37.58 and 34.75% in 60 DAS respectively over no fertilizers at treatment applied in 0 days after sowing.

EBFA and EBFM increased root length 29.76 and 32.68% in treatment applied in 20 DAS, 33.97, 35.52% in treatment applied in 20 +40 DAS and 34.76 and 35.58% treatment applied in 40 DAS respectively over no fertilizers. EBFM and EBFA treated plants increased root length as compare to free form of bio fertilizers (FBF) in different mode of application of treatment. Treatment twice applied in 20 + 40 DAS increased root length over other treatments (applied in 0 DAS, 20 DAS, and 20+ 40 DAS and 40 DAS).

Organic matrix entrapped Biofertilizers bind with acacia (EBFA) and molasses (EBFM) increased shoot length 37.70 and 39.89% in 20 DAS, 30.13 and 42.35% in 40 DAS, 43.90 and 42.70% in 60 DAS respectively over no fertilizers at treatment applied in 0 days after sowing. EBFA and EBFM increased shoot length 42.35 and 32.80% in treatment applied in 20 DAS, 38.36 and 47.23% in treatment applied in 20 +40 DAS and 44.74 and 46.33% treatment applied in 40 DAS respectively over no fertilizers after 60 days after sowing.

No. of root and leaves

Organic matrix entrapped Bio-fertilizers bind with acacia (EBFA) and molasses (EBFM) increased no. of leaves 27.24 and 19.81% in 20 DAS, 37.52 and 44.50% in 40 DAS, 22.16 and 29.98% in 60 DAS respectively over no fertilizers at treatment applied in 0 days after sowing. EBFA and EBFM increased no. of leaves 17.63 and 26.22% in treatment applied in 20 DAS, 12.38 and 26.22% in treatment applied in 20 +40 DAS and 12.38 and 22.16% treatment applied in 40 DAS respectively over no fertilizers after 60 days after sowing.

Organic matrix entrapped Biofertilizers bind with acacia (EBFA) and molasses EBFM) increased no. of roots 46.19 and 22.13% in 20 DAS, 58.33 and 60.24% in 40 DAS, 21.43 and 24.19% in 60 DAS respectively over no fertilizers at treatment applied in 0 days after sowing. EBFA and EBFM increased no. of roots 12.00 and 26.70% in treatment applied in 20 DAS, 21.44 and 29.54% in treatment applied in 20 +40 DAS and 15.46 and 21.43% treatment applied in 40 DAS respectively over no fertilizers after 60 days after sowing.

Dry weight of root and shoot

Organic matrix entrapped Biofertilizers bind with acacia (EBFA) and molasses EBFM) increased dry wt. of roots 28.57 and 16.66% in 20 DAS, 23.07 and 47.36% in 40 DAS, 66.29 and 23.06% in 60 DAS respectively over no fertilizers at treatment applied in 0 days after sowing. EBFA and EBFM increased fresh wt. of roots 42.30 and 38.46% in treatment applied in 20 DAS, 45.45 and 44.44% in treatment applied in 20 +40 DAS and 43.39 and 40% treatment applied in 40 DAS respectively over no fertilizers after 60 days after sowing.

Organic matrix entrapped Biofertilizers bind with acacia (EBFA) and molasses EBFM) increased dry wt. of shoots 12.00 and 22.33% in 20 DAS, 87.48 and 85.48% in 40 DAS, 12.08 and 28.75% in 60 DAS respectively over no fertilizers at treatment applied in 0 days after sowing. EBFA and EBFM increased dry wt. of shoots 54.28 and 40.29% in treatment applied in 20 DAS, 71.22 and 47.01% in treatment applied in 20 +40 DAS and 87.82 and 87.53% treatment applied in 40 DAS respectively over no fertilizers after 60 days after sowing.

Wheat Yield

Organic matrix entrapped Biofertilizers bind with acacia (EBFA) and molasses EBFM) increased grain yield 45.94% and 39.39% respectively over no fertilizers at treatment applied in 0 days after sowing.

EBFA and EBFM increased grain yields 23.07% and 33.33% in treatment applied in 20 DAS, 48.71% and 36.66% in treatment applied in 20 +40 DAS and 13.04 and 16.66% treatment applied in 40 DAS respectively over no fertilizers after 60 days after sowing (Figure 1).

Level of Nitrate, Nitrite, Ammonium and phosphate content in soil, roots and leaves applied different mode of application at 20 and 60 DAS

Nitrate, Nitrite, Ammonium and phosphate content in soil, root and leaves of wheat crop were increased in treatment of organic matrix entrapped biofertilizers bind with acacia and molasses in compare to other treatment at different mode of treatment. Treatment twice applied in 20 and 40 DAS increased nitrogenous component in compare to other applied treatment at 20 and 40 days after sowing.

Level of nitrate content in soil, roots and leaves applied different mode of application in 20 and 60 DAS

Organic matrix entrapped Biofertilizers bind with acacia (EBFA) and molasses EBFM) increased nitrate content in soil 6.58 and 8.50% in 20 DAS and 4.40 and 9.06% in 60 DAS at treatment applied in 0 DAS, 12.52 and 10.04% in 20 DAS and 8.87 and 6.28% in 60 DAS at treatment applied in 20 DAS, 12.27 and 14.28% in 20 DAS, 2.92 and 6.98% in 60 DAS at treatment applied in 20 +40 DAS, 19.69 and 22.13% in 20 DAS, 1.84 and 4.73% in 60 DAS at treatment applied in 40 DAS respectively over no fertilizers (NF) (Figure 2).

Organic matrix entrapped Biofertilizers bind with acacia (EBFA) and molasses EBFM) increased nitrate content in fresh root of wheat plant 58.67 and 62.82% in 20 DAS and 56.42 and 59.86% in 60 DAS at treatment applied in 0 DAS, 66.38 and 69.33% in 20 DAS and 46.19 and 54.96% in 60 DAS at treatment applied in 20 DAS, 56.50 and 57.44% in 20 DAS, 70.71 and 72.99% in 60 DAS at treatment applied in 20 +40 DAS, 28.08 and 34.19% in 20 DAS, 34.05% and 48.15% in 60 DAS at treatment applied in 40 DAS respectively over no fertilizers (NF) (Figure 2).

Organic matrix entrapped Biofertilizers bind with acacia (EBFA) and molasses EBFM) increased nitrate content in fresh leaves of wheat plant 44.75 and 45.41% in 20 DAS and 37.21 and 34.75% in 60 DAS at treatment applied in 0 DAS, 29.70 and

31.23% in 20 DAS and 28.58 and 33.97% in 60 DAS at treatment applied in 20 DAS, 23.02 and 21.39% in 20 DAS, 26.86 and 13.15% in 60 DAS at treatment applied in 20 + 40 DAS, 21.14 and 19.08% in 20 DAS, 9.22% and 7.27% in 60 DAS at treatment applied in 40 DAS respectively over no fertilizers (NF) (Figure 2).

Level of nitrite content in soil, roots and leaves applied different mode of application in 20 and 60 DAS

Organic matrix entrapped Biofertilizers bind with acacia (EBFA) and molasses EBFM) increased nitrite content in dry soil of wheat plant 55 and 53.84% in 20 DAS and 34.88 and 40.42% in 60 DAS at treatment applied in 0 DAS, 50.81 and 39.79% in 20 DAS, 51.72 and 48.14% in 60 DAS at treatment applied in 20 DAS, 62.50 and 48.57% in 20 DAS, 50.00 and 47.16% in 60 DAS at treatment applied in 20 +40 DAS, 50 and 51.35% in 20 DAS, 66.03% and 73.58% in 60 DAS at treatment applied in 40 DAS respectively over no fertilizers (NF) (Figure 3).

Organic matrix entrapped Biofertilizers bind with acacia (EBFA) and molasses EBFM) increased nitrite content in fresh root of wheat plant 48.48 and 51.42% in 20 DAS and 34.14 and 56.52% in 60 DAS at treatment applied in 0 DAS, 51.42 and 54.05% in 20 DAS, 73.68 and 73.33% in 60 DAS at treatment applied in 20 DAS, 54.05 and 58.53% in 20 DAS, 28.57 and 70.14% in 60 DAS at treatment applied in 20 +40 DAS, 51.42 and 52.77% in 20 DAS, 51.21% and 58.33% in 60 DAS at treatment applied in 40 DAS respectively over no fertilizers (NF) (Figure 3).

Table.1 Treatment design

Treatment	Form of fertilizer	Days	Doses
1.	No fertilizers	---	-----
2.	Free form(Azoto +PSB) in seed	0-days	single
3.	Free form (azoto + PSB)	0-dsay	single
4.	Immobilized form with acacia (saresh plant gum)	0-days	single
5.	Immobilized form with mollasses	0-days	single
6.	Free form (azoto + PSB)	20-days	single
7.	Immobilized form with acacia (saresh plant gum)	20-days	single
8.	Immobilized form with molasses	20-days	single
9.	Free form (azoto + PSB)	20+40-days	single
10.	Immobilized form with acacia (saresh plant gum)	20+40-days	single
11.	Immobilized form with mollasses	20+40-days	single
12.	Free form (azoto + PSB)	40-days	single
13.	Immobilized form with acacia (saresh plant gum)	40-days	single
14.	Immobilized form with mollasses	40-days	single

Table.2 Effect of different mode of application on growth of wheat (*Triticum aestivum* L. cv. PBW-343) root length and Shoot length on 20, 40, 60 days after sowing. All the values are means of three replicates (n=3) ± S.D. (Where NF= No fertilizers, SCBF= Seed coated biofertilizers, FBF= Free form of biofertilizers, EBFA= Organic matrix entrapped biofertilizers with binder Acacia, EBFM- Organic matrix entrapped biofertilizers with binder Mollasses

Root length	**Treatment applied in 0 DAS**			**Treatment applied in 20 DAS**		
	20 DAS	**40 DAS**	**60 DAS**	**20 DAS**	**40 DAS**	**60 DAS**
NF	2.17±0.31	4.17±0.35	5.50±0.50			
SCBF	3.33±0.29	5.67±1.04	7.00±2.00			
FBF	3.83±0.29	5.83±1.61	8.33±0.58	3.00±0.31	5.83±0.29	7.50±0.50
EBFA	5.23±0.93	6.17±1.04	8.81±0.60	3.47±0.50	7.50±0.50	7.83±1.04
EBFM	5.10±0.17	6.00±1.80	8.43±0.51	4.40±0.90	7.43±0.40	8.17±1.26
	Treatment twice applied in 20 + 40 DAS			**Treatment applied in 40 DAS**		
	20 DAS	**40 DAS**	**60 DAS**	**20 DAS**	**40 DAS**	**60 DAS**
FBF	3.33±0.29	6.10±0.66	8.00±1.73	2.67±0.58	7.17±0.29	6.83±0.76
EBFA	3.80±0.26	7.50±0.50	8.33±0.29	2.83±0.76	7.33±0.76	8.43±0.40
EBFM	4.73±0.87	7.67±0.76	8.53±0.45	2.83±1.89	7.67±0.76	8.50±0.50
Shoot length	**Treatment applied in 0 DAS**			**Treatment applied in 20 DAS**		
	20 DAS	**40 DAS**	**60 DAS**	**20 DAS**	**40 DAS**	**60 DAS**
NF	15.43±2.60	25.27±3.73	31.13±1.18			
SCBF	25.67±0.76	29.10±0.53	36.67±7.02			
FBF	24.50±0.87	36.17±5.80	51.67±7.64	26.20±0.85	28.17±1.15	47.83±5.75
EBFA	24.77±2.89	36.17±5.35	55.50±6.06	23.87±1.00	29.33±3.69	54.00±8.19
EBFM	25.67±2.52	43.83±1.89	54.33±1.15	22.33±1.53	35.17±4.25	46.33±9.02
	Treatment twice applied in 20 + 40 DAS			**Treatment applied in 40 DAS**		
	20 DAS	**40 DAS**	**60 DAS**	**20 DAS**	**40 DAS**	**60 DAS**
FBF	20.67±3.75	29.67±2.02	47.33±2.52	22.00±1.00	36.67±7.11	44.00±4.58
EBFA	23.17±2.89	32.17±3.33	50.50±7.37	20.83±4.31	32.83±1.61	56.33±8.50
EBFM	21.50±0.50	36.17±3.75	59.00±7.76	21.00±0.50	37.33±2.31	58.00±2.12

Table.3 Effect of different mode of application on growth of wheat (*Triticum aestivum* L. cv. PBW-343) number of roots and number of Leaves on 20, 40, 60 days after sowing. All the values are means of three replicates (n=3) ± S.D. (Where NF= No fertilizers, SCBF= Seed coated biofertilizers, FBF= Free form of biofertilizers, EBFA= Organic matrix entrapped biofertilizers with binder Acacia, EBFM- Organic matrix entrapped biofertilizers with binder Mollasses

Root number	**Treatment applied in 0 DAS**			**Treatment applied in 20 DAS**		
	20 DAS	**40 DAS**	**60 DAS**	**20 DAS**	**40 DAS**	**60 DAS**
NF	2.33±0.58	3.33±0.58	7.33±0.58			
SCBF	4.00±1.00	7.33±1.53	8.67±1.53			
FBF	3.33±0.58	6.33±1.53	9.00±1.00	3.67±1.53	7.00±1.00	8.67±0.58
EBFA	4.33±1.15	8.00±1.00	9.33±1.53	3.00±5.00	8.33±0.58	8.33±0.58
EBFM	3.00±1.00	8.33±0.58	9.67±0.58	4.00±1.00	8.67±0.58	10.00±1.00
	Treatment twice applied in 20 + 40 DAS			**Treatment applied in 40 DAS**		
	20 DAS	**40 DAS**	**60 DAS**	**20 DAS**	**40 DAS**	**60 DAS**
FBF	3.33±0.58	7.67±0.58	8.33±0.58	3.67±0.58	7.33±0.58	8.33±0.58
EBFA	3.33±0.5 8	8.67±0.58	9.33±2.31	3.67±0.58	7.67±0.58	8.67±0.58
EBFM	4.00±1.00	9.33±0.58	10.33±0.58	4.00±1.00	8.00±1.00	9.33±0.58

Leave number	**Treatment applied in 0 DAS**			**Treatment applied in 20 DAS**		
	20 DAS	**40 DAS**	**60 DAS**	**20 DAS**	**40 DAS**	**60 DAS**
NF	2.67±0.58	3.33±0.58	4.67±0.58			
SCBF	2.67±0.577	5.33±0.58	7.00±1.00			
FBF	3.33±0.577	5.67±0.58	6.00±1.00	3.33±0.577	5.33±0.58	6.33±0.58
EBFA	3.67±0.577	5.33±0.58	6.00±1.00	3.67±0.577	5.67±0.58	5.67±1.15
EBFM	3.33±0.577	6.00±0.00	6.67±0.58	4.00±1.000	4.67±0.58	6.33±0.58
	Treatment twice applied in 20 + 40 DAS			**Treatment applied in 40 DAS**		
	20 DAS	**40 DAS**	**60 DAS**	**20 DAS**	**40 DAS**	**60 DAS**
FBF	3.33±0.58	5.17±0.76	5.67±1.15	3.33±0.58	5.33±0.58	5.67±1.53
EBFA	3.67±0.58	5.67±1.15	5.33±1.53	4.00±1.00	4.67±0.58	5.33±0.58
EBFM	4.00±1.00	6.00±0.00	6.33±0.58	3.00±0.00	4.33±0.58	6.00±1.00

Table.4 Effect of different mode of application of bio fertilizers on root and shoot dry weight of

wheat (*Triticum aestivum* L. cv. PBW-343), (All the values are means of three replicates (n=3) ± S.D.), (Where NF= No fertilizers, SCBF= Seed coated biofertilizers, FBF= Free form of biofertilizers, EBFA= Organic matrix entrapped biofertilizers with binder Acacia, EBFM- Organic matrix entrapped biofertilizers with binder Mollasses

Root dry weight	**Treatment applied in 0 DAS**			**Treatment applied in 20 DAS**		
	20 DAS	**40 DAS**	**60 DAS**	**20 DAS**	**40 DAS**	**60 DAS**
NF	0.005±0.002	0.010±0.010	0.030±0.026			
SCBF	0.006±0.002	0.068±0.038	0.247±0.219			
FBF	0.007±0.002	0.031±0.004	0.046±0.043	0.005±0.002	0.018±0.002	0.027±0.012
EBFA	0.007±0.002	0.013±0.005	0.089±0.007	0.006±0.003	0.044±0.032	0.050±0.007
EBFM	0.006±0.001	0.019±0.002	0.039±0.013	0.004±0.003	0.035±0.030	0.052±0.010
	Treatment twice applied in 20 + 40 DAS			**Treatment applied in 40 DAS**		
	20 DAS	**40 DAS**	**60 DAS**	**20 DAS**	**40 DAS**	**60 DAS**
FBF	0.005±0.002	0.024±0.015	0.050±0.042	0.006±0.003	0.033±0.040	0.012±0.003
EBFA	0.005±0.001	0.013±0.011	0.055±0.011	0.015±0.011	0.007±0.002	0.053±0.004
EBFM	0.004±0.002	0.007±0.001	0.054±0.005	0.008±0.003	0.006±0.001	0.042±0.010
Shoot dry weight	**Treatment applied in 0 DAS**			**Treatment applied in 20 DAS**		
	20 DAS	**40 DAS**	**60 DAS**	**20 DAS**	**40 DAS**	**60 DAS**
NF	0.08±0.010	0.12±0.036	0.08±0.015			
SCBF	0.08±0.065	0.089±0.269	0.028±0.065			
FBF	0.013±0.059	0.05±0.221	0.17±0.038	0.103±0.043	0.306±0.050	0.103±0.043
EBFA	0.091±0.006	0.09±0.050	0.091±0.006	0.075±0.002	0.902±0.044	0.175±0.002
EBFM	0.103±0.054	0.08±0.106	0.103±0.054	0.034±0.025	0.940±0.054	0.134±0.025
	Treatment twice applied in 20 + 40 DAS			**Treatment applied in 40 DAS**		
	20 DAS	**40 DAS**	**60 DAS**	**20 DAS**	**40 DAS**	**60 DAS**
FBF	0.139±0.037	0.347±0.005	0.139±0.037	0.571±0.412	0.290±0.044	0.571±0.412
EBFA	0.278±0.322	0.375±0.083	0.278±0.322	0.057±0.035	0.912±0.067	0.657±0.035
EBFM	0.151±0.011	0.841±0.071	0.151±0.011	0.042±0.030	0.721±0.102	0.642±0.030

Table.5 Post Hoc Test Duncan[a.b.c].in multiple range of different parameter

S. No.	**Parameter**	**DAS**	**N**	**Subset**		
				1	**2**	**3**
1	**Root length**	1.00	14	3.6229[a]	6.7543[b]	7.8707[c]
2	**Shoot length**	2.00		22.6864[a]	33.4271[b]	49.4729[c]
3	**No. of root**	3.00		3.5471[a]	7.5686[b]	8.9514[c]
4	**No. of leaf**	Sig.		3.4286[a]	3.7879b	5.9286[c]
5	**Root fresh wt.**			0.0293[a], 0.0664[a]	5.1786[b]	
6	**Root dry wt.**			0.0064[a], 0.0234	0.4086[b]	
7	**Shoot fresh wt.**			0.2529[a], 0.4650a	0.0604[b]	
8	**Shoot dry wt.**			0.1294[a],0.2329[a]	0.4329[b]	

Value within each column followed by the same letter are not significantly different ($p < 0.05$) using Duncan's Multiple Range Test)

Fig.1 Grain yield (gm/plant) of *Triticum aestivum L.* at harvesting stages in different mode of application (A= Treatment applied in 0 days; B= Treatment applied in 20 days after sowing; C= Treatment twice applied in 20 + 40 days after sowing; D= Treatment applied in 40 days after sowing)

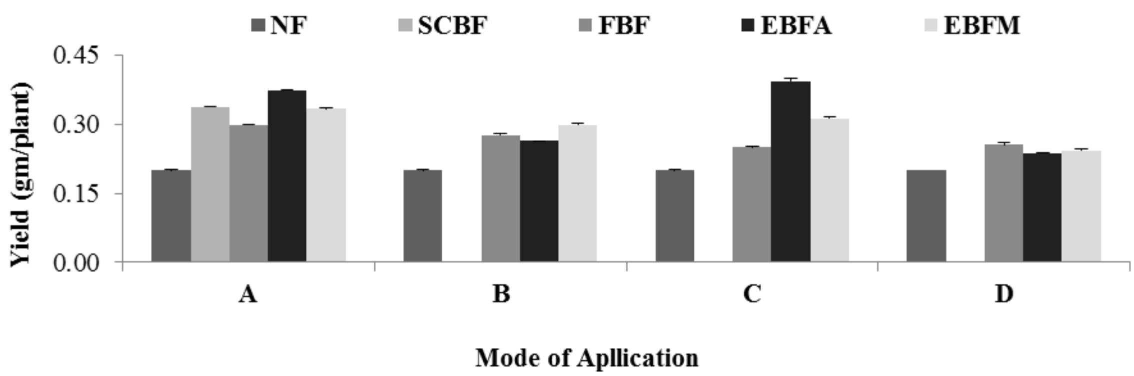
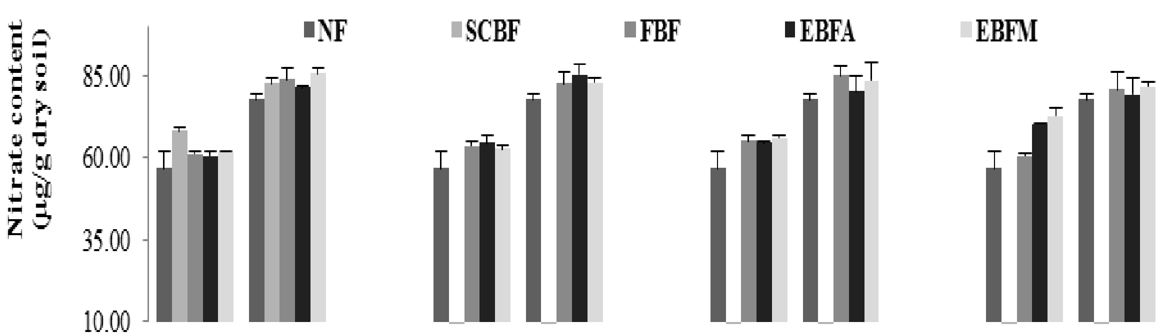


Fig.2 Concentration of Nitrate (µg/g) in soil, root and leaves of *Triticum aestivum L.* at 20 DAS and 40 DAS in different mode of treatment (A= Treatment applied in 0 days; B= Treatment applied in 20 days after sowing; C= Treatment twice applied in 20 + 40 days after sowing; D= Treatment applied in 40 days after sowing)



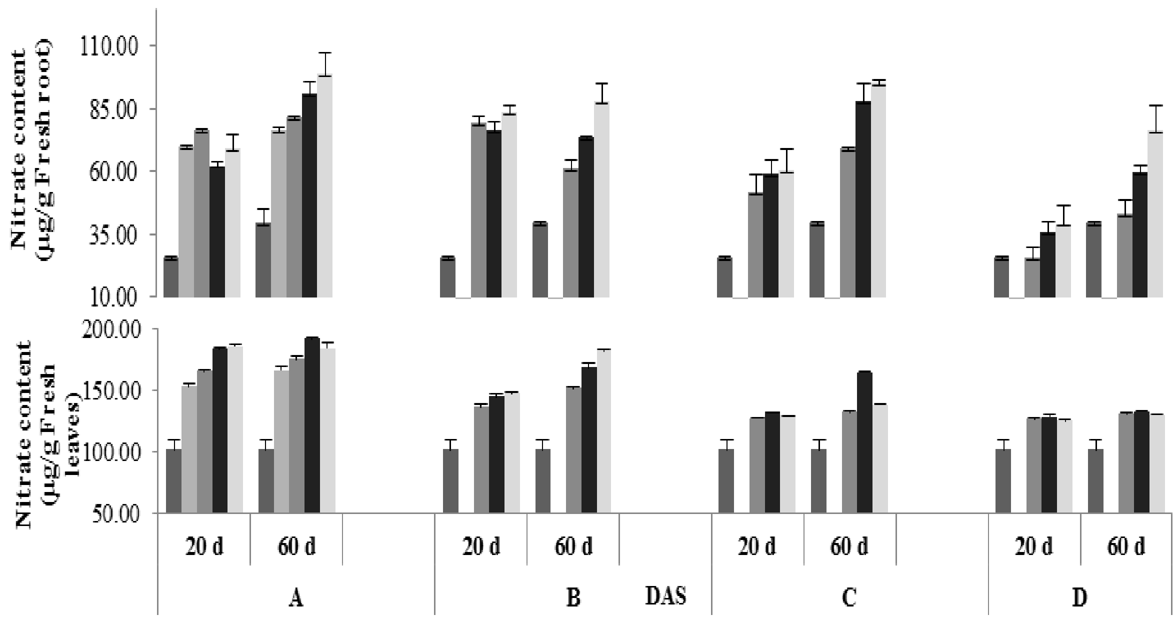


Fig.3 Concentration of Nitrite (µg/g) in soil, root and fresh leaves of *Triticum aestivum L.* at 20 DAS and 40 DAS in different mode of treatment (A= Treatment applied in 0 days; B= Treatment applied in 20 days after sowing; C= Treatment applied in 20 + 40 days after sowing; D= Treatment applied in 40 days after sowing)

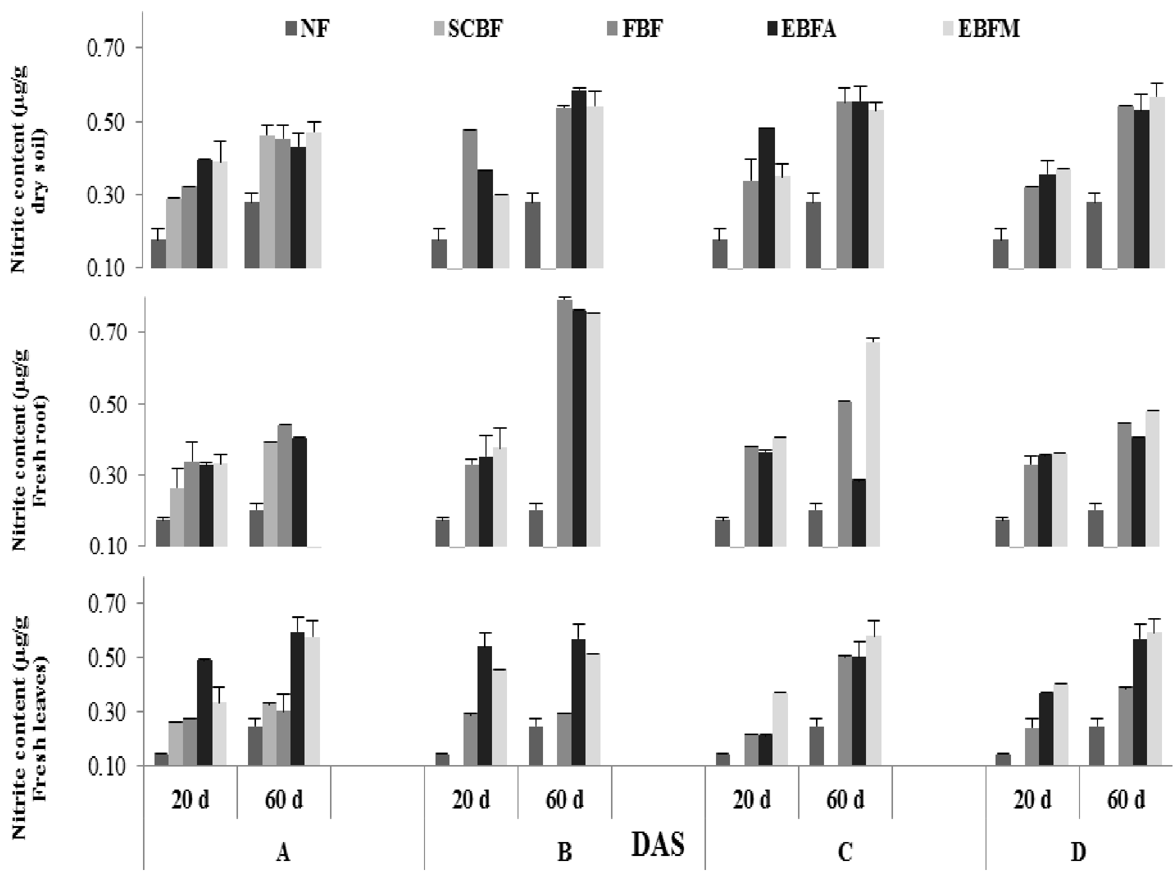


Fig.4 Concentration of Ammonium (µg/g) in soil, root and fresh leaves of *Triticum aestivum L.* at 20 DAS and 40 DAS in different mode of treatment (A= Treatment applied in 0 days; B= Treatment applied in 20 days after sowing; C= Treatment applied in 20 + 40 days after sowing; D= Treatment applied in 40 days after sowing)

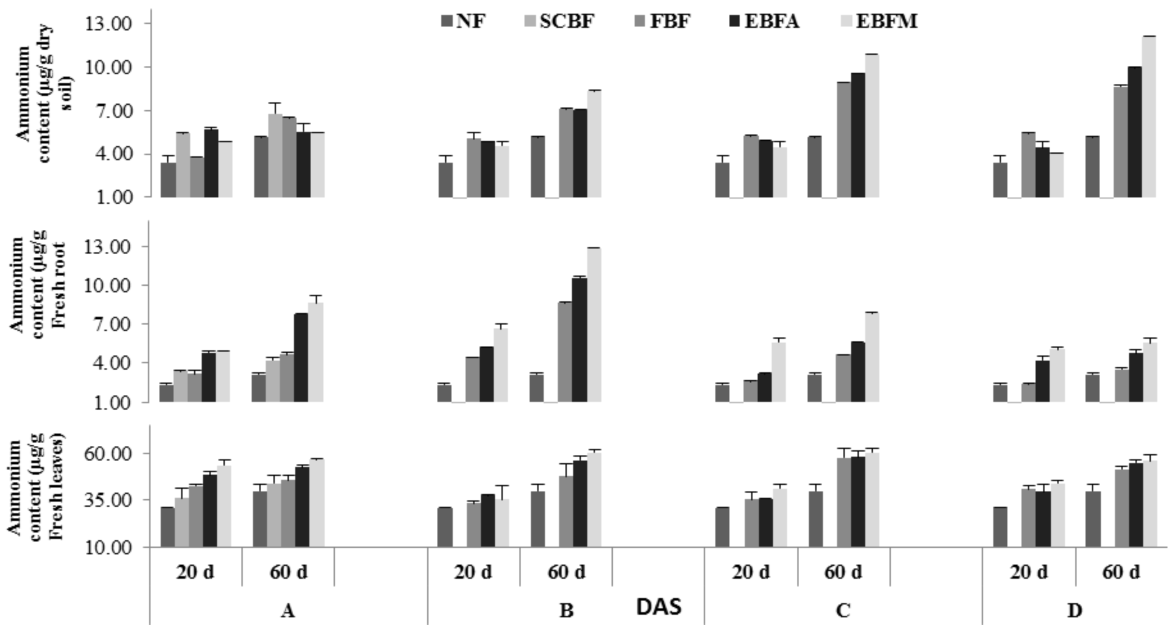
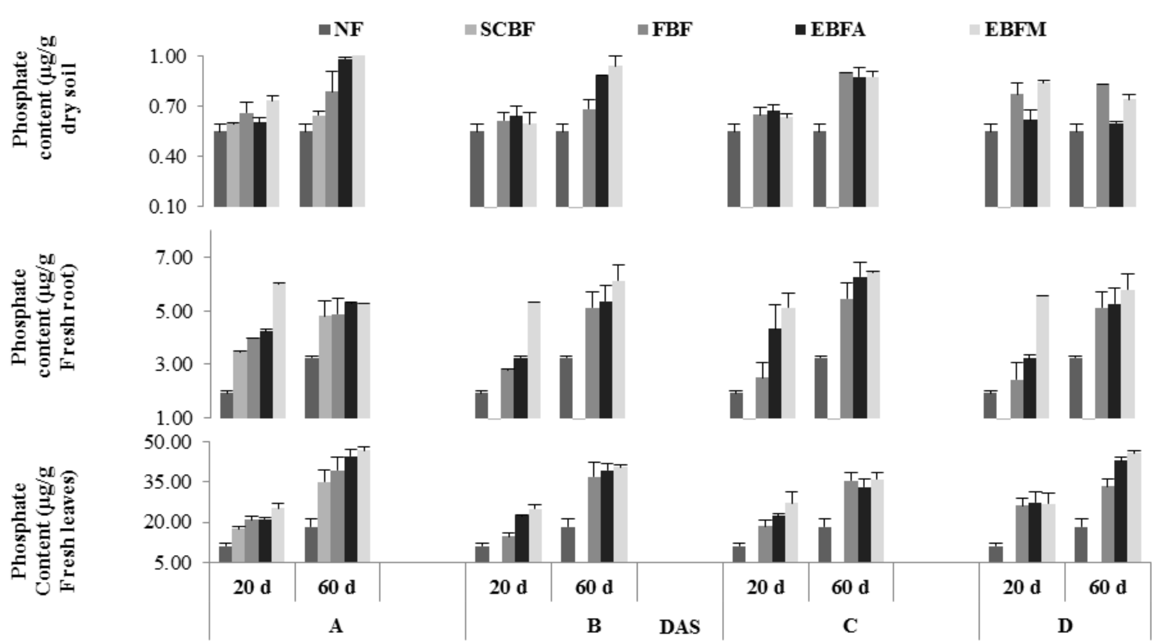


Fig.5 Concentration of Phosphate (µg/g) in soil, root and fresh leaves of *Triticum aestivum L.* at 20 DAS and 40 DAS in different mode of treatment (A= Treatment applied in 0 days; B= Treatment applied in 20 days after sowing; C= Treatment applied in 20 + 40 days after sowing; D= Treatment applied in 40 days after sowing)



Organic matrix entrapped Biofertilizers bind with acacia (EBFA) and molasses EBFM) increased nitrite content in dry soil of wheat plant 71.42 and 57.57% in 20 DAS and 60 and 58.62% in 60 DAS at treatment applied in 0 DAS, 74.07 and 68.88% in 20 DAS, 57.14 and 52.94% in 60 DAS at treatment applied in 20 DAS, 33.33 and 62.16% in 20 DAS, 52.00 and 58.62% in 60 DAS at treatment applied in 20 +40 DAS, 62.16 and 65% in 20 DAS, 57.89% and 59.32% in 60 DAS at treatment applied in 40 DAS respectively over no fertilizers (NF) (Figure 3).

Level of ammonium content in soil, roots and leaves applied different mode of application in 20 and 60 DAS

Organic matrix entrapped Biofertilizers bind with acacia (EBFA) and molasses EBFM) increased Ammonium content in dry soil of wheat plant 40.28 and 30.45% in 20 DAS and 7.05 and 5.29% in 60 DAS at treatment applied in 0 DAS, 30.45 and 25.38% in 20 DAS, 27.19 and 38.36% in 60 DAS at treatment applied in 20 DAS, 31.02 and 24.38% in 20 DAS, 46.23 and 52.62% in 60 DAS at treatment applied in 20 +40 DAS, 23.70 and 16.54% in 20 DAS, 48.39% and 49.20% in 60 DAS at treatment applied in 40 DAS respectively over no fertilizers (NF) (Figure 4).

Organic matrix entrapped Biofertilizers bind with acacia (EBFA) and molasses EBFM) increased Ammonium content in fresh root of wheat plant 51.55 and 52.43% in 20 DAS and 60.23 and 64.00% in 60 DAS at treatment applied in 0 DAS, 55.08 and 64.86% in 20 DAS, 70.60 and 75.86% in 60 DAS at treatment applied in 20 DAS, 26.18 and 35.18% in 20 DAS, 44.56 and 60.48% in 60 DAS at treatment applied in 20 +40 DAS, 26.65 and 38.77% in 20 DAS, 34.93% and 44.26% in 60 DAS at treatment applied in 40 DAS respectively over no fertilizers (NF) (Figure 4).

Organic matrix entrapped Biofertilizers bind with acacia (EBFA) and molasses EBFM) increased Ammonium content in fresh leaves of wheat plant 35.47 and 42.21% in 20 DAS and 23.95 and 29.61% in 60 DAS at treatment applied in 0 DAS, 16.90 and 12.06% in 20 DAS, 29.21 and 33.91% in 60 DAS at treatment applied in 20 DAS, 13.55 and 24.57% in 20 DAS, 31.45 and 33.99% in 60 DAS at treatment applied in 20 +40 DAS, 21.54 and 29.45% in 20 DAS, 27.53% and 28.89% in 60 DAS at treatment applied in 40 DAS respectively over no fertilizers (NF) (Figure 4).

Level of Phosphate content in soil, roots and leaves applied different mode of application in 20 and 60 DAS

Organic matrix entrapped Biofertilizers bind with acacia (EBFA) and molasses EBFM) increased Phosphate content in dry soil of wheat plant 8.33 and 24.65% in 20 DAS and 39.79 and 43.26% in 60 DAS at treatment applied in 0 DAS, 14.06 and 6.77% in 20 DAS, 37.44 and 36.55% in 60 DAS at treatment applied in 20 DAS, 17.91 and 12.69% in 20 DAS, 36.78 and 35.86% in 60 DAS at treatment applied in 20 +40 DAS, 11.66 and 34.52% in 20 DAS, 1.66% and 20.27% in 60 DAS at treatment applied in 40 DAS respectively over no fertilizers (NF) (Figure 5).

Organic matrix entrapped Biofertilizers bind with acacia (EBFA) and molasses EBFM) increased Phosphate content in fresh root of wheat plant 54.50 and 68.05% in 20 DAS and 38.37 and 37.90% in 60 DAS at treatment applied in 0 DAS, 40.92 and 63.77% in 20 DAS, 39.29 and 46.81% in 60 DAS at treatment applied in 20 DAS, 55.76 and 62.42% in 20 DAS, 47.68 and 49.14%

in 60 DAS at treatment applied in 20 +40 DAS, 40.55 and 65.46% in 20 DAS, 38.14% and 43.59% in 60 DAS at treatment applied in 40 DAS respectively over no fertilizers (NF) (Figure 5).

Organic matrix entrapped Biofertilizers bind with acacia (EBFA) and molasses EBFM) increased Phosphate content in fresh leaves of wheat plant 47.74 and 56.15% in 20 DAS and 59.40 and 61.34% in 60 DAS at treatment applied in 0 DAS, 50.80 and 55.92% in 20 DAS, 48.97 and 55.30% in 60 DAS at treatment applied in 20 DAS, 50.47 and 59.19% in 20 DAS, 45.34 and 49.80% in 60 DAS at treatment applied in 20 +40 DAS, 59.67 and 59.10% in 20 DAS, 57.77% and 60.48% in 60 DAS at treatment applied in 40 DAS respectively over no fertilizers (NF) (Figure 5)

Wheat, a member of family Poaceae, is an important cereal for staple food. It plays an important role in human nutrition and agriculture economy of country. The production and productivity of wheat can be increased by using good fertilizers and developing new varieties with high yield potential and stability. Our findings will provide significant insight in understanding the behavior of these bio-fertilizer microbes in a given matrix based micro-environment, which have been used by us very effectively to prepare granular slow release fertilizers for some cereals, oil crops, a legume and vegetable crops leading to high productivity and yield as well as better soil enrichment. The entrapped biofertilizers are expected to be more effective than the free biofertilizers due to a given organic rich and porous (airy) micro-environment for their multiplication and activities.

The loading of nitrogen based chemical fertilizers e.g. Urea, NPK etc. has been reported to gain high yield and productivity in the cereals including wheat. The excessive use of inorganic fertilizers is unsustainable for any farming practice from economic as well as ecological points of view, the use of various kinds manure including microbial inoculants (biofertilizers) for fixing nitrogen, solubilizing phosphates and decomposing / recycling carbon have been studied (22, 24). Bio- fertilizers are live microbial system such as cyanobacteria, other nitrogen fixing bacteria (*Rhizobium*, *Azotobacter*, *Azospirillum* etc.), phosphate solubilizing bacteria (*Bacillus* etc.), Fe and S mobilizing bacteria etc., which of chemical fertilizers, water and pesticide etc.

Biofertilizers are important not only for the reduction of quality of chemical fertilizers but also for getting better yield in sustainable agriculture. Soil micro-organisms play a key role in soil phosphorous dynamics and subsequent availability of phosphate to plants. Nutrient availability in the soil-plant system is dictated by complex interactions between plant roots, soil micro-organisms, chemical reactions and pathways of losses.

The concentrations dependents of most of the processes that nutrients undergo in soil include transformations induced by microbes (N_2 fixation, nitrification, denitrification, immobilization etc.), chemical process (exchange, fixation, precipitation, hydrolysis, etc. (14).

Thus, the inherent supply to the liable pool in soil is inadequate to meet production goals. This situation demands for developing strategies and policies to boost NPK supplies in a timely manner and in sufficient quantities and for developing technologies to increase fertilizers use efficiency. One such strategy is the use of slow release fertilizers (SRFs).

Nitrogen is one of the major mineral nutrients required for growth and development of plants, and plant growth is often dependent upon nitrogen supply. It has also been shown that when factors others than nitrogen supplies are limiting growth; there is dependence between nitrogen uptake and the relative growth during N starvation. Furthermore, the availability of different forms of nitrogen and health land communities confers advantages to the root surface to utilize nitrogen (14). It is generally recognized that, although plants may take up nitrogen in various forms, the main source in natural condition are nitrate and ammonium ions. Nitrate is the key form of the nitrogen supply for cereal plants.

The applied nitrogen can be lost through nitrate leaching, resulting in contamination of ground and surface water (1), volatization of ammonia, gaseous losses of NOx, building greenhouse gases and global warming etc. (2, 11).

The use of slow release or control release fertilizers to reduce nitrogen loss from agricultural land and raise nitrogen fertilizers efficiency has been recommended (27). However, little information is available on the impact of such fertilizers on NO_3^- leaching, runoff losses, and NH_3 volatilization in crop field. On account of its controlled release character, coated urea always resulted in a lower nutrient concentration in water, so nitrogen loss was small at each stage.

The results indicate that the biofertilizers provide better nutrient availability and crop productivity. The entrapment of these biofertilizers to a biodegradable, low cost organic matrix contained local and cheap agro-waste materials like cow-dung, neem leaf powder, clay soil binder Acacia gum (saresh) and molasses, enhanced its efficacy over the free form of biofertilizers. These fertilizers are applied in different mode of application i.e. treatment applied in 0 days, applied in 20 days after sowing, twice applied in 20 and 40 days after sowing and 40 days after sowing increased the growth parameter and nitrogenous component in the wheat plants.

Our results indicate that mode of application of bio-fertilizers affect the growth, productivity and yield of wheat and availability, translocation of nutrients e.g. (Nitrate, Nitrite, Ammonium and Phosphate) in rhizospheric soil, roots and leaves. The mixing of these biofertilizers with organic matrix prepared with clay soil, cow dung, neem leaves entrapped with acacia and molasses. The main effect of the saresh and molasses as a binder to entrapped the organic matrix (Clay soil, cow dung, neem leaves with consortium of Biofertilizers (*Azotobacter*, *Bacillus*) gave the best formulations to increase root length, shoot length, no of leaves, and dry wt. of roots and shoots as applied in different mode of treatments i.e. treatment applied in 0 days, applied in 20 days after sowing, twice applied in 20 and 40 days after sowing and 40 days after sowing. These formulations also increased the availability of inorganic nitrogen species (nitrate, nitrite and ammonium) and phosphate in rhizosphere (0-10 cm.) of wheat and in wheat leaves by consortium of biofertilizers. The mode of application was indicate that the consortium of biofertilizers provide better nutrient availability and crop productivity.

In addition, entrapment of these biofertilizers to a biodegradable, low cost organic matrix contained local and cheap agro-waste materials like cow-dung, neem leaf powder, clay soil and acacia gum saresh and molasses enhanced its efficacy over the free form of biofertilizers and no fertilizers.

References

1. Adesemoye AO, Torbert HA, and Kloepper JW. Plant growth promoting rhizobacteria allow reduced application rates of chemical fertilizers. Microbiol Ecol 2009; 58: 921–929.
2. Akiyama H, Tsuruta H, and Watanabe T. N_2 O and NO emissions from soils after the application of different chemical fertilizers. Chemosphere-Global Chan. Sci 2000; 2: 313-320.
3. Bakht J, Shafi M, Jan MT and Shah Z. Influence of Crop Residue Management, Cropping System and N Fertilizers on Soil N and C Dynamics and Sustainable Wheat (*Triticum aestivum* L.) production. Soil Till Res 2009; 104: 233-240.
4. Broschat TK and Moore KK. Release of ammonium-nitrogen, nitrate nitrogen phosphorus, potassium, magnesium, iron and manganese from seven controlled release fertilizer. Comm. Soil Sci. Plant Anal 2007; 38: 843-850.
5. Cataldu DA, Haroon M, Schvander LE and Young L. Rapid Colorimetric Determination of Nitrate in Plant Tissue by Nitrification of Salicylic Acid. Comm. Soil Sci. Plant Analy 1975; 6: 71-80.
6. Dahiya S, Jaiwal PK and Singh RP. Efficient Nitrogen Assimilation and High Productivity in Rice (*Oryza sativa* L.) Applied with Organic Matrix Based Slow Release Nitrogen Fertilizers. Physiol. Mol. Biol Plant 2004; 10: 83-92.
7. Emilsson T, Berndtsson JC, Mattsson JE and Rolf K. Effect of Using Conventional and Controlled Release Fertilizer on Nutrient Runoff from Various Vegetated Roof Systems. Ecol Eng 2007; 29: 260-271.
8. Gopinath KA, Saha S, Mina BL, Pande H, Kundu S and Gupta HS. Influence of Organic Amendments onGrowth, Yield and Quality of Wheat and onSoil Properties during Transition to Organic Production. Nutr Cycling Agro 2008; 82: 51-60.
9. Granta CA, Wub R, Selles F, Harkerc KN, Claytond GW, Bittmane S, Zebarthf BJ and Lupwayi NZ. Crop Yield and Nitrogen Concentration with Controlled Release Urea and Split Applications of Nitrogen as Compared to Non-coated Urea Applied at Seeding. Field Crop Res 2012; 127: 170-180.
10. Heitkamp F, Raupp J and Ludwig B. Soil Organic Matter Pools and Crop Yields as Affected by the Rate of Farmyard Manure and Use of Biodynamic Preparations in a Sandy Soil. Organic Agri Sci 2011; 1: 111-124.
11. Jiang J, Hu Z, Sun W and Huang Y. Nitrous Oxide Emissions from Chinese Cropland Fertilized with a Range of Slow Release Nitrogen Compounds. Agri. Eco. Environ 2010; 135: 216-225.
12. Joshi AK, Mishra EB, Chatrath ER, Ferrara GO and Singh RP. Wheat Improvement in India: Present Status, Emerging Challenges and Future Prospects. Euphytica 2007; 157: 431–446.
13. Kumar M, Bauddh K, Sainger M, Sainger PA, Singh JS and Singh RP. Increase in Growth, Productivity and Nutritional Status of Rice (*Oryza sativa* L.cv. Basmati) and Enrichment in Soil Fertility Applied with an Organic Matrix Entrapped Urea. J. Crop Sci Biotechnol 2012; 15(2): 137-144.
14. Kumar S, Bauddh K, Barman SC and Singh RP. 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. J Agr Sci Tech 2014; 16: 315-329.

15. Kundu BS, Nehra K, Yadav R and Tomar M. Biodiversity of Phosphate Solubilizing Bacteria in Rhizosphere of Chickpea, Mustard and Wheat Grown in Different Region of Haryana. Ind J Microbiol 2009; 49: 120-127.
16. Mahajan A, Chaudhary AK, Jaggi RC and Dogra RK. Importance of Biofertilizers in Sustainable Agriculture. Farm Forum 2003; 17-20.
17. Ogut M, Akdag C, Duzdemir O and Sakin MA. Single and Double Inoculation with Azospirillum/Trichoderma: The Effects on Dry Bean and Wheat. Biol Fert Soils 2005; 41: 262–272.
18. Rawat SK, Singh RK and Singh RP. Seasonal Variation of Nitrate Level in Ground and Surface Waters of Lucknow and Its Remediation Using Certain Aquatic Macrophytes. Int J Lakes Riv 2010; 3(1): 25-35.
19. Sharma P, Singh G and Singh RP. Conservation Tillage, Optimal Water and Organic Nutrients Supply Enhanced Soil Microbial Activity during Wheat (Triticum aestivum) Cultivation. Brazil J Microbiol 2011; 42: 531-542
20. Sharma VK and Singh RP. Organic Matrix Based Slow Release Fertilizers Enhances Plant Growth, Nitrate Assimilation and Seed Yield of Indian Mustard (*Brassica juncia* L.). J Environ Biol 2011; 32: 619-624.
21. Shaukat K, Affrasayab S and Hasnain S. Growth Responses of *Triticum aestivum* to Plant Growth Promoting Rhizobacteria Used as a Biofertilizers. Res J Microbiol 2006; 1(4): 330-338.
22. Shekoofa A and Emam Y. Effects of Nitrogen Fertilization and Plant Growth Regulators (PGRs) on Yield of Wheat (*Triticum aestivum* L.) cv. Shiraz. J Agric Sci Technol 2008; 10: 101-108.
23. Singh RP, Dahiya S and Jaiwal PK. Slow Release Fertilizers for Sustained Nitrogen Supply and High Plant Productivity. In: "Nitrogen Nutrition in Plant Productivity", (Eds.): Rana P. Singh, Shankar, N. and Jaiwal, P. K. Studium Press, LLC, Houston, Texas, USA, PP. 2006; 329-349.
24. Singh RP, Kumar M and Jaiwal PK. Improvement in Nitrogen Use Efficiency and Yield of Crop Plants by Sustained Nutrient Supply and Enhanced Nitrogen Assimilation. In: "Development in Physiology, Biochemistry and Molecular Biology of Plants", (Eds.): Bose, B and Hemantranjan, A. New India Publishing Agency, New Delhi 2008a; 2: 1-31.
25. Singh RP, Sainger M, Bauddh K, Sengar RS and Jaiwal PK. Sustained Nutrient Supply Reduced Nutrient Loss and High Plant Productivity with Slow Release Fertilizers, In: "Stable Food Production and Sustainable Agriculture; A Challenge ahead 21st Century" (Eds.): Sengar, R. S. and Sharma, A. K.. Studium Press, Pvt Ltd., India 2010; PP. 62-79.
26. Stevens DL and Oaks A. The Influence of Nitrate in the Induction of Nitrate Reductase in the Maize Roots. Can J Bot 1973; 51: 1255-1258.
27. Vishalakcchi A, Kumar S, Singh RP. Response of Organic Matrix Entrapped biofertilizers on Growth, Yield and soil properties of Rice (*Oryza sativa* L.) Asian Journal of

Agriculture and Food Science 2014; 02: 2321 – 1571.

28. Weatherburn MW. Phenol-hypo Chlorite Reaction for Determination of Ammonia. Anal Chemist 1967; 39: 971-974.
29. Weligama C, Sale PWG, Conyers MK, Liu DL and Tang C. Nitrate Leaching Stimulates Subsurface Root Growth of Wheat and Increase Rhizosphere Alkalization in a Highly Acidic Soil. Plant Soil 2010; 328: 119:132.
30. Wu L, Liu M and Liang R. Preparation and Properties of a Double Coated Slow Release NPK Compound Fertilizer with Super Absorbent and Water Retention. Bioresour Technol 2008; 92: 547-554.
31. Wu SC, Cao ZH, Li ZG, Cheung KC and Wong MH. Effects of Biofertilizers Containing N-fixer, P and K Solubilizers and AM Fungi on Maize Growth: A Green House Trial Geoderma 2005; 125: 155-166.
32. Zhao GZ, Liu YQ, Tian Y, Sun YY and Cao Y. Preparation and Properties of Macro molecular Slow-release Fertilizers Containing Nitrogen, Phosphorus and Potassium. J Polymer Res 2010; 17:119-125.