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BIOFERTILIZER: A NOVEL FORMULATION FOR IMPROVING WHEAT GROWTH, PHYSIOLOGY AND YIELD

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Abstract

Bacillus cereus and *Pseudomonas moraviensis* strains were inoculated singly as well as in consortium with two different carriers i.e., maize straws and sugarcane husk in the formulation of biofertilizer. Plant growth promoting rhizobacteria (PGPR) strains used in biofertilizer were phosphate solubilizer and exhibited strong antifungal activities**.** Both PGPR used in formulation was maintained 15–16.5×10^8 cfu g^{-1} in carrier material after 40d. The field experiment was conducted at Quaid-e-Azam University Islamabad on wheat for two consecutive years (2011-2012) simultaneously in pots and field. Plants sampling for growth and physiological parameters was made after 57d of sowing and at maturity for yield parameters. Single inoculation of *Pseudomonas moraviensis* and *Bacillus cereus* with maize straw and sugarcane husk increased plant height and fresh weight by 18-30% and protein, proline, sugar contents and antioxidant activities by 25-40%. There were 20% increases in spike length, seeds/spike and seed weight in single inoculation. Co-inoculation of PGPR further increased plant growth, physiology and yield by 10-15% over single inoculation with carriers. PGPR consortium with sugarcane husk and maize straw (biofertilizer formulation) increased 20-30% plant growth chlorophyll, sugar, protein contents, antioxidants activities and yield parameters. It is inferred that carrier based biofertilzer effectively increased growth, maintained osmotic balance and enhanced the activities of antioxidant enzymes and yield parameters.

Key words: Sugarcane husk in agriculture, *Pseudomonas moraviensis*, *Bacillus cereus*.

Introduction

The global food production has increased by many folds as compared to past (Pretty, 2008) but still pace of agriculture productivity is not adequate for rapidly increasing population. Presently, chemical fertilizers used for crop yield improvements are not compatible because of their environmental hazards and cost. In present scenario, there has been a real resurgence of environmental friendly, sustainable agricultural products (Orhan *et al*., 2006).

Biofertilizers are defined as the formulations containing living microorganisms or latent cells having the potential of colonizing roots of crops plants and promote the growth by improving nutrients availability and acquisition (Packialakshmi & Aliya, 2014).

Plant growth promoting rhizobacteria (PGPR) are basic components of a biofertilizer. The PGPR strains such as *Burkholderia*, *Azospirillium*, *Enterobacter, Azotobacter*, *Erwinia, Rhizobium* and *Flavobacterium* have proved prolific in this regard (Rodriguez & Fraga, 1999). These Plant growth promoters improved the availability of nutrients (N, P, Zn and Fe), and increased production of phytohormones (categorised as phytostimulators) (Naveed *et al*., 2008). The representatives of *Bacillus* and *Pseudomonas* are highly potent bio-inoculants of cereals (Talik *et al*., 2006).

Formulations of biofertilizers are made by the combination of effective microorganisms and carrier-based inoculants. Carriers are advantageous C-sources for incorporation of microorganisms, because they are capable of improving effectiveness of biofertilizers by elongating the shelf life (Stephens & Rask, 2000; Ferreira & Castro; 2005). Bacterial inoculants including rhizobia, nitrogen-fixing rhizobacteria (Franche *et al*., 2009), plant growth-promoting rhizobacteria (Podile & Kishore, 2006), phosphate-solubilizing bacteria are sources of growth promotion in these bioformulations (Marchner, 1995).

A good carrier material should be non-toxic to inoculant bacterial strains and plants, good moisture absorber, easy to process and sterilize, inexpensive and easily available. Good adhesion to seeds, good pH and buffering capacity are also recommended for carriers. Rice husk, farm yard manure (FYM), Charcoal, peat and lignite have been proven as good carrier material (Mahdi *et al*., 2010).

Bacillus subtilis and *Pseudomonas corrugate* strains were inoculated with different carriers (alginate beads, alginate beads + skim milk and charcoal) to improve root and shoot length and dry weight of maize (Trivedi *et al.,* 2005).

Biofertilizers and phytostimulators have been frequently hampered due to unavailability of accurate consortia and the variability and inconsistency of results between laboratory, greenhouse, and field studies (Artursson *et al*., 2006). Temperature, pH, humidity, soil nature, native microflora and insect population also halt the effectiveness of biofertilizers (Lucy *et al*., 2004).

Biofertilizers have long been assessed by exploring native and non-native PGPR but this is the first study of its kind to evaluate the effects of biological wastes products (sugarcane husk and maize straws) as carriers. Additionally, the use of two novel strains i.e., *Bacillus cereus* and *Pseudomonas moraviensis* as growth, physiology and yield promoter of wheat in different combinations with two carriers was focused.

Material and Methods

Soil preparation and sowing: Two isolates *Pseudomonas moraviensis* (accession No. LN714047) and *Bacillus cereus* (accession No. LN714048) isolated from rhizosphere soil of *Cenchrus ciliaris* were applied on wheat

under pots and field condition for two consecutive years. Liquid culture of *Bacillus cereus, Pseudomonas moraviensis* and were prepared by growing PGPR in L.B media for 7d.

The soil in the field was ploughed and plots measuring 5 m^2 with row to row distance 20 cm were prepared. No chemical fertilizer was added but adequate N, P and K were available in soil. Treatment comprised of Maize straw (MZ), sugarcane husk (SC), *Pseudomonas moraviensis* (PM) + MZ as carrier), *Pseudomonas moraviensis* + SC as carrier), *Bacillus cereus* (BC+ MZ), *Bacillus cereus* (BC) + SC), consortium (PM+BC), (BC+PM with MZ), (BC+PM + SC) and un-inoculated control (C). For each treatment four replicates were used. Seeds coated with biofertilizer were sown by hand drill method.

Method of biofertilzer preparation and application

Preparation of inocula: Liquid cultures of *Bacillus cereus* and *Pseudomonas moraviensis* were prepared by growing these PGPR in L.B media for 7d (10^8cfu/ml and O.D ~ 1 at 600 nm). The co-inoculation of two PGPR was made on the basis of synergistic behaviour of two PGPR strains (data not presented).

Formulation of biofertilizer: Maize straw and sugarcane husk were shade dried at room temperature under sterilized condition and sieved through 0.20-0.31 mm sieve (ANTAI China). The Carrier (50g); previously sterilized by autoclaving was inoculated with 20ml of liquid broth having ($8.5–9.9\times10^9$ cells ml^{-1}cfu) with 1% molasses. Product thus formed was incubated for 24h in laminar flow, packed and sealed.

Inoculation studies: Surface sterilized seeds (treated with 70% ethanol and distilled water) of wheat var. Inqlab-91 were used for experiment. The seeds were successively washed with autoclaved distilled water. The biofertilizer (2 g) was mixed with 100ml autoclaved water which was sufficient for 250 g seeds. Seeds were soaked for 1h, shade dried for 15 min prior to sowing. The average temperature of the growing area was 25 ± 2°C with 11h photo period and 13 h dark period; humidity varied from 75-80%.

P-solublization potential of PGPR: Pure colonies of bacterial strains were pin point inoculated on sterilized Pikovskaya agar (Pikovskaya, 1948). Appearance of halo zones around colonies indicated phosphate solubilizing ability (Vyas *et al*., 2007). Bacterial colonies were isolated from 10^7 dilution and were inoculated separately into conical flasks containing Pikovskayas broth and incubated at room temperature (25±2ºC) on an orbital shaker for 6d. Both bacterial cultures were centrifuged at 8000g for 20 min at room temperature (25±2ºC) and 2 ml aliquots of the supernatant were used to detect soluble phosphorous using chloromolybdic acid-stannous chloride method (Jackson, 1967) at 882 nm. The soluble phosphate was calculated from a standard curve of KH_2PO_4.

Antifungal activity of PGPR: For the determination of antifungal activities of bacterial extracts, agar tube dilution method was used (Washington & Sutter, 1980). The capacity of each strain to inhibit fungal growth (%) was determined as;

$$\text{Fungal growth inhibition (\%)} = \frac{\text{100 - Linear growth in test sample (mm)}}{\text{Linear growth in control (mm).}} \text{ x 100}$$

Sampling and Physico-chemical analysis of soil: Rhizosphere soil samples of wheat were collected at early vegetative stage (57DAS) below 7-10 cm from surface. Soil samples were homogenized, and sieved through 2mm sieve and processed for the isolation of rhizobacteria and determination of physico-chemical properties. Soil pH was measured by preparing 1:1(soil: water) suspension as explained by (McKeague, 1978; Mclean, 1982), while soil organic matter was determined by method of Walkley & Black, (1934).

Nitrate (NO_3-N) and Phosphorus (P) content of soil: Soil Nitrate-N (NO_3-N) and Phosphorus (P), were extracted by the method of Reitemeier (1943).

Protein contents of leaves: For all physiological parameters leaves of 57 d old plants were used. For soluble protein estimation, method of Lowry *et al*., 1951was followed. Leaf tissue (0.1 g) were ground in phosphate buffer (pH 7.5) and centrifuged for 10 min at 3000 g. 0.1 ml of supernatant was diluted with distilled water to make final volume (1 ml). After addition of Folin Phenol reagent, absorbance was recorded at 650 nm.

Sugar (glucose) estimation: Glucose content of leaves were measured by Dubo *et al*., (1983) which was modified by Johnson *et al*., (1966).Homogenate (0.5 g plant tissue + 10ml distilled water) was centrifuged at 3000 g for 5 min. Supernatant (0.1ml) was reacted with 5ml concentrated sulphuric acid. Samples were incubated for 4 h and analysed at 420 nm for measuring the change in absorbance.

Proline estimation: Leaves proline content were estimated by the method of Bates *et al*. (1973). Plant tissue (0.5 g) was grinded with 3% sulphosalicylic acid (10 ml). Homogenate was filtered through Whatman No. 42 filter paper and 2 ml of filtrate was added with 2 ml acid ninhydrin with subsequent addition of 2ml of glacial acetic acid. Test tubes containing mixture were placed in water bath for 1 h at 100°C. Thereafter, 4ml of toluene was used to extract and toluene layer was separated by separating funnel. The absorbance of toluene layer was measured at 520 nm using against toluene as blank.

Antioxidant assay: Extraction of antioxidant enzymes was done by the method of Vetter *et al*. (1958). The assay mixture for peroxidase comprised 1.35ml of 100 mM MES buffer (pH 5.5), 0.1ml enzyme extract, 0.05% H_2O_2 and 0.1% phenylenediamine. Spectrophotometer

(UV-120-01, Shimadzu) was used to read the change in absorbance at 485 nm. The activity of POD was expressed as ΔOD 485nm/min/ mg protein. The activity of superoxide dismutase (SOD) was measured by the inhibition in photochemical reduction of nitrobluetetrazolium (NBT) as demonstrated by Beauchamp & Fridovich, (1971).

Determination of phytohormones: Phytohormones (IAA, GA and ABA), extractions was done by the method of Kettner & Doerffling, (1995). Wheat leaves (1g) were ground in 80% methanol with an antioxidant, butylated hydroxyl toluene (BHT) at 4°C. Laves were extracted for 72 h at 4°C in dark with subsequent change of methanol. Obtained extract was centrifuged at 3000 g for 5 min at 4°C. Thereafter, supernatant was reduced to aqueous phase with the help of rotary thin film evaporator. The aqueous phase was partitioned four times using ½ volume of ethyl acetate after adjusting pH to 2.5-3.0. The solvent (ethyl acetate) was evaporated by rotary thin film evaporator. Dried material was re-dissolved in 1ml of HPLC grade methanol (100%). HPLC (Shimadzu, C-R4A Chromatopac; SCL-6B system controller) equipped with C-18 column (39x300mm) and UV detector was used for the detection of hormones. A millipore filter (0.45µm) was injected in the column to filter the samples of 100µl. Pure ABA, IAA and GA_3 (Sigma Aldrich, USA) was used as standard for identification and quantification of hormones. ABA, GA and IAA were identified on the basis of retention time and peak area of standards. Methanol, acetic acid and water (30: 1: 69) were used as mobile phase. The flow rate was adjusted at 0.5 ml/min with an average time for 22 min/sample. The detection of IAA was made at 280 nm (Sarwar *et al*., 1992) and ABA was detected at 260 nm (Dobrev *et al*., 2005). For GA analysis wavelength was also adjusted at 254 nm (Li *et al*., 1994).

Cost benefit ratio analysis: The benefit cost ratio per hectare was calculated by formula explained by Mehmood *et al*., 2011.

BCR = Value of gross production – cost of inputs (investments)/ cost of inputs (investments)
The cost of inputs = $C_{sd} + C_{fert} + C_{carr} + C_{pp} + C_{lab} + C_{land} + C_{irrig} + C_{Misc}$
where
C_{sd} = cost on seed
C_{fert}= cost on fertilizer
C_{carr} = cost on carrier (labour + grinding + transportation)
C_{pp} = cost on plant protection
C_{lab} = cost on labour
C_{land} = cost on land preparation
C_{irrig} = cost on irrigation

Statistical analyses: For statistical analyses Statistix program, version 8.1 was used and data was analyzed for analysis of variance (ANOVA). In field experiments Randomize Block Design (RCBD) was followed and for pots experiment Complete Randomized Design (CRD) was applied. Mean values were separated (p=0.05) and represented by different letters both in tables and figures along with ± standard error.

Results

In association with maize straw and sugarcane husk, cfu of *Bacillus cereus* was 16% and 24% higher than of *Pseudomonas moraviensis* respectively at early vegetative stage (57DAS), in rhizosphere soil of pots grown plants (Fig. 1).

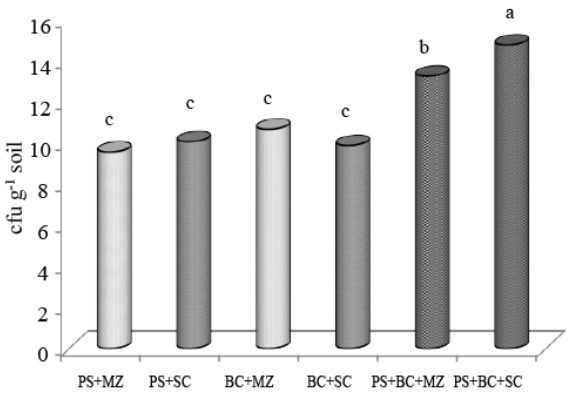

Fig. 1. Colony forming unit(cfu g^{-1} soil) of PGPR applied with carriers in rhizosphere of wheat grown in pots. Measurements were made at 57DAS. MZ = Maize straw powder, SC = Sugarcane husk, PS = *Pseudomonas moraviensis*, BC = *Bacillus cereus*

The organic matter (Table 1) was significantly higher (58% and 29%) in wheat rhizosphere soil of pots and field grown plants when *Pseudomonas moraviensis* was inoculated with maize straw and sugarcane husk. Similarly, inoculation of *Bacillus cereus* with carriers enhanced 82% and 40% organic matter in potted and field grown plants. Percent increases in organic matter were 96% and 50% higher in consortium treatment for potted and field grown plants. Application of PGPR consortium with maize straw and sugarcane husk (biofertilizer treatment) further increased 75% and 30% organic matter in potted and field grown plants over PGPR consortium.

Pseudomonas moraviensis and *Bacillus cereus* inoculation with maize straws and sugarcane husk increased NO_3-N contents of rhizosphere soil by 55-60% and 25% over control in potted and field grown plants respectively (Table 1). This increase was 69% and 35% higher over control in potted and field grown plants when PGPR were co-inoculated. The highest NO_3-N was observed in biofertilzer treatments where 90-100% and 45% greater increase in potted and field grown plants was recorded.

Pseudomonas moraviensis and *Bacillus cereus* inoculation with maize straw and sugarcane husk increased P contents over control by 30-40% in pot grown plants and field grown plants (Table 1). Co-inoculation increased P contents by 45% in potted and field grown plants. Biofertilizer treatment resulted 77% and 65% increase in P contents of potted and field grown plants, respectively.

The K content were 40% higher in pots grown plants and 25% in field grown plants (Table 1) when *Bacillus cereus and Pseudomonas moraviensis* were inoculated singly with carriers. This increase was even higher 56% and 29% in potted and field grown plants respectively.

Biofertilzer treatment further increased K contents by 20% in potted plants.

Table 1. Effects of biofertilizer on organic matter (%) nutrients contents (mg/kg) of rhizosphere soil of wheat grown in pots and field. Measurements were made at 57DAS. Values are mean of four replicates.

Treatments	Pots				Field			
	O. M	NO_3-N	P	K	O.M	NO_3-N	P	K
Control	0.45	14.42	4.96	102.21	0.567	22.43	4.71	77.41
MZ	0.52	15.55	5.24	108.43	0.574	22.98	4.85	80.88
SC	0.5	14.92	5.32	110	0.581	23.22	4.98	81.06
PS + MZ	0.71*	22.22*	6.5*	142.22*	0.712	27.11*	6.08*	94.74*
PS + SC	0.78*	22.91*	6.61*	144.62*	0.724	27.72*	6.19*	96.66*
BC + MZ	0.76*	22.29*	6.48*	146.41*	0.777	27.66*	6.33*	96.31*
BC + SC	0.82*	22.84*	6.9*	149.19*	0.792	28.09*	6.48*	95.23*
PS + BC	0.88*	24.42*	7.11*	159.12*	0.852*	30.21*	6.95*	99.72*
PS + BC + MZ	1.22*	28.62*	8.78*	179.16*	1.09*	32.44*	7.87*	105.63*
PS + BC + SC	1.19*	27.79*	8.39*	182.2*	1.04*	33.14*	7.74*	106.66*

*****Significant at the 0.05 probability level

MZ = Maize straw powder, SC = Sugarcane husk, PS = *Pseudomonas moraviensis*, BC = *Bacillus cereus*

Table 2. Effects of biofertilizer on nutrients acquisition (mg/kg) of wheat leaf grown in pots and field. Measurements were made at 57DAS. Values are mean of four replicates.

Treatments	Pots			Field		
	NO_3-N	P	K	NO_3-N	P	Ca
Control	4.49	3.31	13.38	6.22	5.3	16.46
MZ	5.02	3.44	13.71	6.7	5.8	17.3
SC	5.1	3.58	13.94	6.82	5.91	17.52
PS + MZ	8.55*	6.09*	20.28*	9.5*	7.43*	21.21*
PS + SC	9.06*	6*	21.55*	9.76*	7.62*	21.23*
BC + MZ	8.72*	5.88*	25.21*	9.64*	7.77*	21.66*
BC + SC	9.14*	6.13*	24.94*	9.33*	7.82*	21.56*
PS + BC	9.56*	6.4*	26.66*	12.45*	8.94*	24.87*
PS + BC+MZ	12.12*	7.11*	30.12*	13.44*	9.92*	26.26*
PS + BC + SC	12.44*	7.06*	29.65*	14.06*	9.64*	27.12*

*****Significant at 0.05 probability level

MZ = Maize straw powder, SC = Sugarcane husk, PS = *Pseudomonas moraviensis*, BC = *Bacillus cereus*

The uptake of nutrients (NO_3-N, P and K) in wheat leaf of potted and field grown plants were 80-90% and 40-50% higher than control when *Pseudomonas moraviensis* and *Bacillus cereus* were inoculated alone with sugarcane husk and maize straw (Table 2). PGPR consortium increased 115% NO_3-N and 65% P over control both in potted and field grown plants. Similarly, K contents were increased by 99% in pots and 53% in field grown plants respectively when PGPR were co-inoculated. Biofertilizer treatment showed 64% and 25% further increase in term of NO_3-N in potted and field grown plants respectively. The uptake of P and K was further improved by 18-25% and 35% over single inoculation in potted and field grown plants respectively.

Inoculation of *Bacillus cereus* and *Pseudomonas moraviensis* with sugarcane husk and maize straw, increased plant height by 28-33% in pots (Table 3) and 23-34% in field grown plants (Table 4). Coinoculation of both PGPR increased plant height significantly by 53% and 39% in potted and field grown plants respectively. These increase were also depicted in fresh weight where 29% and 44% increse were recorded in potted and field grown plants. Maximum increases in height (58% and 47%) were observed in potted and field grown plants in biofertilzer treatment. Biofertilizer treatment also enhanced fresh weight of plants by 38% in potted and 55-59% in field grown plants.

Protein contents of leaves of pots grown plants was 33-35% higher over control when plants were inoculated with *Bacillus cereus* and *Pseudomonas moraviensis* with carrier materials (Table 3). Coinoculation of both *Bacillus cereus* and *Pseudomonas moraviensis* significantly increased protein contents by 47% and 39% in pots (Table 3) and field grown plants (Table 4) respectively. Application of biofertilizer increased protein contents by 58-62% in potted and 75% in field grown plants.

Sugar contents of leaves were significantly increased by 26-37% in pots grown plants (Table 3), and 28-42% in field grown plants (Table 4) when PGPR were inoculated alone with carriers. The co-inoculation of PGPR increased soluble sugar and proline contents by 55-60% in pots and 40-48% in field. Maximum increase in sugar and proline was recorded in potted and field grown plants, when biofertilizer was applied.

Superoxide dismutase (SOD) and Peroxidase (POD) activities (Fig. 2) were 31% and 45% higher in pots grown plants when *Bacillus cereus* and *Pseudomonas moraviensis* were inoculated alone with carriers. This increase was 56% and 46% in field grown plants. Co-inoculation increased SOD activity by 45-55% and POD activity by 64% in potted and field grown plants SOD activity was 55% and 76% higher in potted and field grown plants when biofertilizer was applied. POD activity was 65% higher over control in biofertilizer treatment both in potted and field.

Table 3. Effects of carrier based biofertilizer on wheat physiology (57DAS) and yield (159DAS) of pots grown plants.

Treatments	Pots experiment								
	Plant height	Fresh weight	Chlorophyll	Protein	Sugar	Proline	Spike length	Seed/ spike	Seed weight
Control	24.5	1.57	47b	126.56e	238	125	6.75	34.5	37.64d
MZ	28.5	1.65	49.4	145.82d	251	137.5	7.35	39.5	39.53*
SC	29.5	1.69	49	145.49d	243	145.5	7.55	40	40.87*
PS+MZ	31.5*	1.77*	53.3	169.56*	297.5*	208*	7.75*	44.5*	44.53*
PS+SC	32.5*	1.71*	54	169.64*	318.5*	234.5*	8*	45.5*	45.59*
BC+MZ	33.5*	1.77*	54.09	171.27*	322.5*	195*	8.05*	46.5*	45.28*
BC+SC	32.5*	1.75*	55	172.22*	348.5*	241.5*	8.05*	47*	45.41*
PS+BC	35.5*	1.77*	56	185.88*	375*	283*	8.45*	49.5*	46.28*
PS+BC+MZ	38*	1.93*	56.55***	197.22*	378.5*	302.5*	8.9*	52*	53.71*
PS+BC+SC	39*	1.99*	57.28***	203.17*	388.5*	317.5*	9.2*	55.5*	52.7*

*****Significant at 0.05 probability level using
MZ = Maize straw powder, SC = Sugarcane husk, PS = *Pseudomonas moraviensis*, BC = *Bacillus cereus*

Table 4. Effects of carrier based biofertilizer on wheat physiology (57DAS) and yield (159DAS) of field grown.

Treatments	Field experiment								
	Plant height	Fresh weight	Chlorophyll	Protein	Sugar	Proline	Spike length	Seed/ spike	Seed weight
control	33.5	1.54	49.0	98.66	267.5	240.25g	8.98	48	47.7
MZ	38	1.84	52.03	107.8	310.25	263.5f	8.93	48.5	51.4
SC	40.25	1.69	52.05	108.99	314	261.5f	8.58	48.7	52
PS+MZ	41.25**	1.73	56.13	112.68	359.03**	288.25**	11.3**	52.5	53.2**
PS+SC	44*	1.94**	56.5	112.49	367.75	292.75**	11.7**	55	54.8**
BC+MZ	45*	2.08**	55.98	124.03	362.5**	301.25**	11.8**	57.8**	54.3**
BC+SC	44*	2.15**	57.28	120.37	382*	308.25**	12.5*	57.3**	54.7**
PS+BC	47.25*	2.22*	57.93	138.24*	397.25*	338.75*	12.8*	60.8**	55.9**
PS+BC+MZ	49.25*	2.39*	59.13	173.42*	419.5*	347.25*	13.5*	63**	58.2**
PS+BC+SC	50.5*	2.45*	58.88	177.56*	417.25*	350.25*	13.7*	62.5**	58.7**

***,** ** Significant at 0.05, 0.01 probability level
MZ = Maize straw powder, SC = Sugarcane husk, PS = *Pseudomonas moraviensis*, BC = *Bacillus cereus*

Single inoculation of PGPR induced 90% indole acetic acid (IAA) in leaves of plants grown under pots and field conditions (Fig. 3). Co-inoculation of PGPR increased 132% IAA in pots and 161% in field grown plants and this increase was three fold higher over control in biofertilzer treatments; both in potted and field grown plants. Gibberellic acid (GA_3) content were three folds higher in pots grown plants and 4 fold higher in field grown plants when *Bacillus cereus* and *Pseudomonas moraviensis* were separately inoculated with carrier. Biofertilizer treatment further increased GA_3 content by 10-26% in pots and 35% in field grown plants over single inoculation.

Number of plant/m^2 counted at maturity were 44-60% higher when *Bacillus cereus* and *Pseudomonas moraviensis* were mixed with maize straws and sugarcane husk in field grown plants (Table 4). Co-inoculation increased number of plants/m^2 by 81% and spike length by 46% . Biofertilizer treatment increased plant/m^2 by 90% , spike length and seeds/ spike by 60% and seed weight in field grown plants. Similarly 37%, 55% and 30% higher spike length, seeds spike and seed weight were recorded in pots grown plants.

Analysis of cost benefit ratio

BCR for field = 1259$ -520$/520 = 1.32

The cost economic benefit ratio reveals that biofertilzer application may increase former benefits by by 32%.

Discussion

The linear increase in colony count of both *Pseudomonas moraviensis* and *Bacillus cereus* indicated positive effects of PGPR and carrier material (Nagesh *et al*., 2013). The boost in cfu of the PGPR in the presence of carrier materials demonstrate higher C-source and better moisture holding capability which referred for the growth of PGPR bio-inoculant (Mahdi *et al*., 2010). The compositional richness of sugarcane husk equipped with monosacchrides, hemicellulose and amino acids enable it as opulent nutritive source for microbial growth (Du Toit *et al*., 1984).

Bacillus cereus and *Pseudomonas* genera are capable of solubilizing phorsphorus (Talik & Reddy, 2006). These PGPR also have antifungal activity which enable them to survive and compete with indigenous microflora. Chang *et al*., (2011) reported that *Bacillus cereus* exhibit strong antifungal activity against different fungal cultures. *Pseudomonas fluorescens* was considered strong antifungal strain against fungal pathogens (Srivastava & shalini, 2008).

Soil nutrients play important role in the growth and survival of plants under various conditions (Cakmakci *et al*., 2007). Increased NO_3 availability due to bifertilizer application in rhizosphere soil concomitant to better growth of plants and subsequent N uptake evidenced by improving existing N pool of soil is depicted by decreased requirement of inorganic N. Over time, depletion of residual soil N imparts serious consequences in crop growth which can be overawed by PGPR application (Malus`a *et al*., 2012).

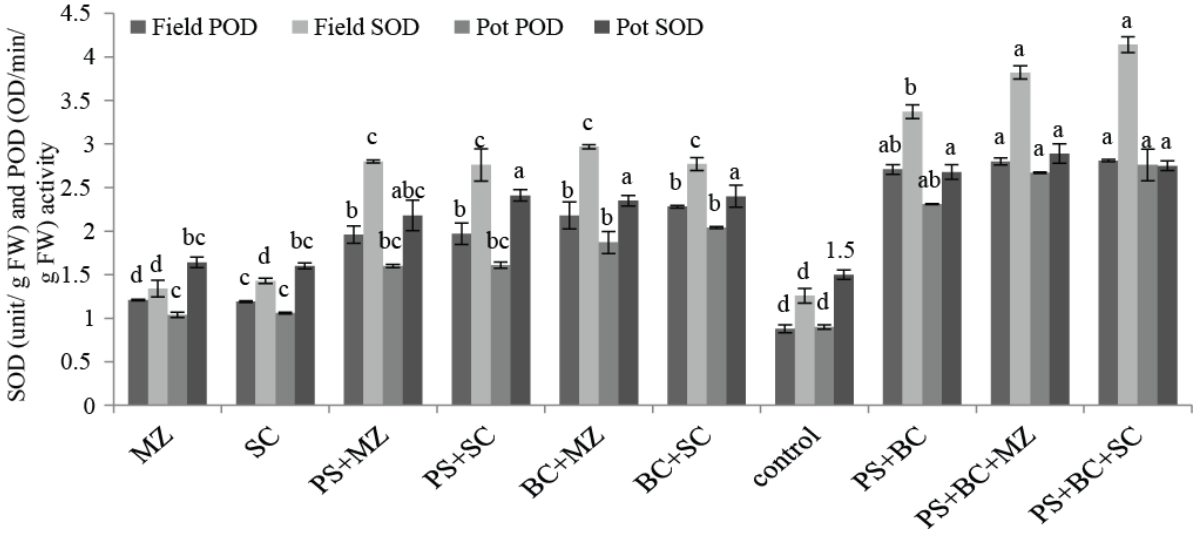

Fig. 2. SOD (units^{-1}g FW) and POD (OD min^{-1} g^{-1} FW) activity of leaves at early vegetative stage of plant growth (after 57d of sowing). Values given are mean of four replicates ± SE. Values followed by different letters heading the bars are significantly different ($p<0.05$) using Statistix 8.1 version. MZ = Maize straw powder, SC = Sugarcane husk, PS = *Pseudomonas moraviensis*, BC = *Bacillus cereus*

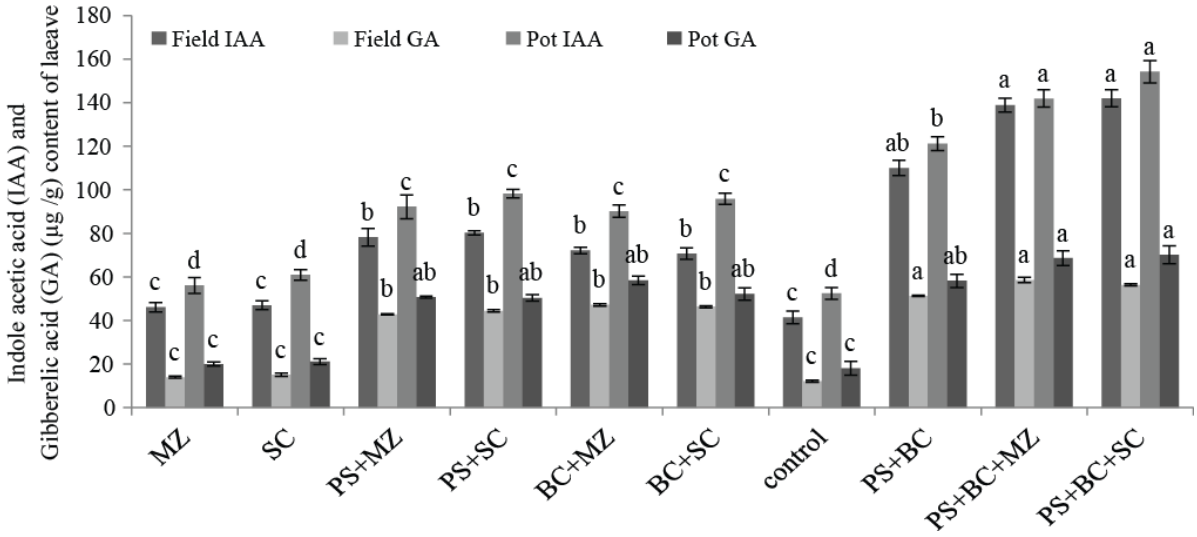

Fig. 3. Indole acetic acid and Gibberellic acid (ug mL^{-1}) contents of leaves at early vegetative stage of plant growth (after 57d of sowing). Values given are mean of four replicates ± SE. Values followed by different letters heading the bars are significantly different ($p<0.05$) using Statistix 8.1 version. MZ = Maize straw powder, SC = Sugarcane husk, PS = *Pseudomonas moraviensis*, BC = *Bacillus cereus*

The positive effect of PGPR applied singly for improving organic matter of rhizosphere soil was noteworthy in the form of biofertilizer (with carriers). Organic matter is essential to improve crop growth and production and soil fertility status because ofrich organic carbon source. Maize straw and sugar cane husk enriched with IAA have been proved a better source of organic matter for improving growth and yield of maize (Mahimairaja *et al*., 2008; Ahmed *et al*., 2011).

Higher accumulation of nutrients in response to PGPR application in apple leaves (Karakurt & Aslantas, 2010). Increase in phosphorus uptake is correlated with the phosphate solublization ability of inoculated PGPR (Aziz *et al*., 2012; Yadav *et al*., 2013). The increase in nutrients contents is correlated with microbial and enzymatic activities and PGPR inoculation further improve the release of nutrients in soil colloidal particles (Gryndler *et al*., 2008; Munir *et al*., 2007). Nutrient uptake and balancing ability in carrier based PGPR formulation might be attributed to the available nutrients of carrier used along with the bio-inoculants (Galal, 2003, Vessey, 2003).

The evidenced increase in plant height and fresh weight might be attributed to better to NO_3, nutrients availability in soil and uptake in treated plants. These results are in agreement with previous verdicts in maize and wheat (Clark *et al*., 1999, Basu *et al*., 2008). Better water holding capacity of the carrier material helped the PGPR to toil efficiently under pots and field conditions as evidenced by significant increase in the fresh weight,

chlorophyll, sugar and proline. Increases in antioxidant enzymes activities may be attributed to the positive role of PGPR on detoxification of reactive oxygen species (Mittova*et al*., 2004; Faize *et al*., 2011).

The improvement in nutrient uptake in plants treated with biofertilizer is correlated with better or higher chlorophyll and protein content. Shah & Ahmad (2006) also reported similar results. Better osmoregulation as evidenced by increase in proline production due to PGPR application appeared as adaptive mechanism (Bashan *et al*., 2004). Enhanced protein contents of leaves with subsequent availability of N-source, increase in growth and metabolism might be due to PGPR application (Akbari *et al*., 2011). The PGPR mediated improvement in soluble sugars and accumulation of carbohydrates in treated plants mediate in maintaining osmotic balance sources (Parida *et al*., 2002; Kumara *et al*., 2009).

Reactive oxygen species (ROS) are major contributor when act as second messenger (Yan *et al*., 2007). Excessive accumulation of ROS is destructive for cells and detoxification, is achieved by enzymyic and non-enzymic antioxidant system under such circumstances. The enzymic antioxidants like catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase are highly active against ROS (Sharma *et al*., 2012). The observed increase in antioxidant super oxide dismutase (SOD) and peroxidase (POD) in treated wheat leaves insinuate toward the better adaptability (Zaefyzadeh *et al*., 2009).

Bacillus and *Pseudomonas* strains have been screened for producing IAA (Spaepen *et al*., 2007). Strains of the same species of *Bacillus cereus and Pseudomonas moraviensis* are proficient in phytohormones production and modulation of IAA, GA and ABA in rhizosphere soil and leaves as recorded in our previous work (Hassan & Bano, 2014). Increase in IAA and GA level in present experiment insinuate the potential of PGPR in improving seed germination, shoot length, root architecture, and fresh weight as documented previously (Hariprasad & Niranjana, 2009; Molina-Favero *et al*., 2008).

Many microbial strains are capable of producing gibberellin (Bottini, 2004). Among *Bacillus* species *Bacillus pumilus* and *Bacillus licheniformis*, showed better growth responses (Gutiérrez-Mañero *et al*., 2001). Previously inoculation of *Azospirillum* sp. and *Bacillus* improved GA contents in rice and wheat (Kucey, 1988). Increased in GA level is correlated with root formation and PGPR like *Azospirillum* spp, *Azotobacter* spp, *Bacillus* spp and rhizobia are known to improve the endogenous level of GA (Dodd *et al*., 2010).

Increase in the seed weight (1000 seeds) might be attributed to improved phosphate solublization ability, indole acetic acid (IAA) and nitrogen availability (Saber *et al*., 2012: Shata*et al*., 2007). Increase in yield of biofertilizer treated plants is correlated with the rhizosphere micoflora which has the ability to promote growth by improving nutrient recycling and absorption (Roesty *et al*., 2006; Shehata & El-Khawas, 2003). The substantial increase in grain yield and grain weight is in agreement with previous findings where biofertilization showed marked increase in yield components of cereals (Dobbelaere *et al*., 2002; Rizwan *et al*., 2008).

Conclusions

It is inferred from the present findings that the PGPR proliferate better in carrier material thereby augmented the shelf life of the PGPR which may seems to protective mechanism of PGPR against desiccations stress, but also provide C/N source as they are rich in organic matter. The presence of carrier material along with consortium of PGPR, acting synergistically appears better solution for sustainable agriculture. Application of PGPR consortium imparted positive effects on physiological and biochemical parameters. *Bacillus cereus* strain was more suitable for improving physiology. Sugarcane husk was a suitable carrier for biofertilzer, and the use of sugarcane husk in biofertilizer may overcome the management problems of sugarcane industry in sustainable manner.

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