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CENCHRUS CILIARIS ROOT POWDER; A SOURCE OF ORGANIC MATTER AND PLANT GROWTH PROMOTING BACTERIA FOR WHEAT

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Abstract

The potential of *Cenchrus ciliaris* L. root powder in sterilized form (as organic matter source) and un-sterilized form with plant growth promoting bacteria (PGPB); *Pseudomonas moraviensis, Bacillus cereus* and *Stenotrophomonas maltophilia* was evaluated for improving soil health, physiology and yield of wheat. *Cenchrus ciliaris* roots were shade dried and ground in powder form. Root powder was applied to wheat in the field, as well as in pots containing sterilized soil at Quaid-e-Azam University Islamabad, for two consecutive years. Pot grown plants comprising 20 g root powder/ pot, while 150 g root powder was added in plot of 1 square meter in field. PGPB existing in applied un-sterilized root powder were re-isolated from rhizosphere soil of pots grown plants after 57d and 122d of sowing. Application of root powder in sterilized form improved organic matter (45%), P and Mg contents of rhizosphere soil and leaves, and positively affected growth, protein content and antioxidant enzymes activities. The application of root powder in un-sterilized form further improved nutrient contents in rhizosphere soil and leaves. Protein, proline, sugar contents, antioxidant activities, Indole acetic acid (IAA), gibberellic acid (GA) and yield components were also improved. The cost benefit ratio analysis for per hectare wheat production reveals that root powder application may increase the farmer's benefit by 19% in field. Root powder may be a rich source of organic matter as well as Phytostimulant for better crop growth and development.

Key words: Grasses and agriculture; Nutrient acquisition; *Stenotrophomonas*; *Pseudomonas moraviensis*.

Introduction

Phyto-stimulation is an advanced phyto-technology. It refers to a technique in which the activities of microflora present in root zone or rhizosphere are enhanced. Plants are interacted with other plants by variety of compounds known as allelochemicals (Saraf *et al*., 2014). Soil organic matter has significant importance for growth of crops. Rhizosphere priming and rhizo-deposition can efficiently alter the soil organic matter (SOM), by improving the decomposition, caused by plant roots activities (Dijkstra *et al*., 2013).

Buffel grass (*Cenchrus ciliaris*), a commonly occurring drought resistant weed is capable of growing in various environments. *C. ciliaris* is a perennial herb of Asian and African regions. The plant reaches to 50 cm in height, bearing spikes at flowering season (Clayton *et al*., 2006). Deep rooting system and higher biomass production facilitate the plant to cope with drought stress (Singariya *et al*., 2012). Additionally, the role of *C. ciliaris* in carbon sequestration, nitrogen cycling and soil binding contributes toward ecosystem stability (Sinha *et al*., 1996).

Exploration and inoculation of PGPB (plant growth promoting bacteria), for improving crops physiology and productivity is contributing in agriculture improvement. Graminaceous crops and prairie plants are effective sources of PGPB (bacterial strains that exist within the plant tissues) (Compant *et al*., 2005). Endophytes are capable of improving growth, physiology and yield of cereals and many agronomical crops have been improved (Ghiyasi *et al*., 2008; Saharan & Nehra, 2011).

The inoculation of PGPB is often accompanied by improved mobilization of nutrients or by P-solubilization and siderophore production (Cakmakci *et al*., 2007). The representatives of *Burkholderia*, *Enterobacter, Azospirillium*, *Azotobacter, Rhizobium, Erwinia* and *Flavobacterium* are considered as competent PGPB. The dominating role of PGPB is particularly associated with the strains belonging to *Bacillus* and *Pseudomonas*. They are potential root colonizers of many plant species, and competent for improving yield indices, due to synergistic behaviour (Hussain & Hasnain, 2009).

B. cereus has been documented as plant growth promoter for its vital antifungal and P-solublisation activities. The application of *B. cereus* as PGPB was effective in enhancing the growth yield and nutrient of broccoli (*Brassica oleracea* var. *italic*) (Yildirim *et al.,* 2006). The effective role of *B. cereus* as bio-pesticide in Pigeon Pea has also been reported (Rani *et al*., 2011). Similarly, Gutierrez-Luana *et al*., (2010) ascribed the stimulatory effects of *B. cereus* to increase biomass of *Arabidopsis thaliana*.

Pseudomonas spp are administrated for their strong role as PGPB, as evidenced previously, to improve seed germination and yield (Shaukat *et al*., 2006). They are demonstrated to enhance growth, phytohormones and yield of chick pea and sugarcane (Rokhzadi *et al*., 2008). Application of *Pseudomonas* on wheat reveals that population size of inoculum and development phase of crop are important factors (Wachowska *et al*., 2006).

The efficacy of *Stenotrophomonas* as plant growth promoter, biocontrol agent, and antibiotic producer has been documented previously (Taghavi *et al*., 2009; Hayward *et al*., 2010). *B. cereus* has been studied for the promotion of growth of *Allium ascalonicum, Brassica juncea* and wheat (Aziz *et al*., 2012; Hassan *et al*., 2018). Similarly, Yadav *et al*., (2013) demonstrated the role of *P. moraviensis* in IAA production; isolated from wheat rhizosphere. However, most of these studies were limited to lab or green house conditions.

The possibility of effectiveness of PGPB with native sources (roots), to promote cereals growth and physiology has never been explored previously. Since phytoremediation and intercropping are less feasible for cereals, a novel method of PGPB in the form of root powder might be beneficial for improving productivity. Present study was aimed to evaluate the role *C. ciliaris* root powder as organic matter and PGPB source. The effects of root powder were recorded on soil fertility, growth, physiology and yield of wheat under field condition and compared with potted plants grown under axenic condition.

Material and Methods

Plant material and growing condition: The Buffle grass (*Cenchrus ciliaris* L.), a naturally grown herb, was collected when the plants were 15 cm high. The roots were cleaned with sterilized water, shade dried for 5-7 d, and ground into powder form, using Anex grinder KC106. Three bacteria were isolated from root powder. Wheat (*Triticum aestivum* L.) var. Inqlab 91 seeds were obtained from National Agricultural Research centre, Islamabad. Seeds were grown in earthen pots (13 cm in diameter and 18 cm in height), having autoclaved soil. Seeds were also grown in field at Quaid-e-Azam University, Islamabad. Root powder in sterilized (RP) or un-sterilized (RP + PGPB) form was applied both in pot and field grown plants, and compared with untreated control (C). The root powder was applied at the rate of 150 g/ 1 square feet in field by hand drill method. Rows were made at the distance of 36 cm. In pot experiment, root powder was supplemented at the rate of 20 g /pot. After 57 days of sowing (DAS), plants were sampled for physiological parameters. The yield parameters were recorded at maturity (159 DAS).

Seeds were sterilized with 70% ethanol for 5 min and soaked in 10% chlorox (2-3 min). After washing with autoclaved distilled water, seeds were sown.

Isolation of microbes and determination of colony forming unit from root powder and soil: For isolation of bacteria, 1 g root powder was suspended in 9 ml water (autoclaved distilled). LB culture media was inoculated with 100 µl of decimal dilution. Microbial culture plates were incubated at 37ºC for 24-72 h. The numbers of viable cell counts at 10^7 dilutions were calculated following the formula adapted by James (1978). For the determination of cfu/g soil the 10^{-8} dilution was selected and following formula was applied.

$$\text{Viable cell count (cfu/g)} = \frac{\text{Number of colonies}}{\text{Volume of inocula}} \text{ x Dilution factor}$$

Physiological analysis of plants: Protein content of leaves was determined by the method of Lowry *et al*., (1951). Amino acid proline was determined by the method explained by Bates *et al*., (1973). Soluble sugar (glucose) was measured by the method devised by Dubo *et al*., (1956). Antioxidant peroxidase activity (POD) was determined by the method of Vetter *et al*., (1958). Superoxide dismutase (SOD) activity was determined by measuring the inhibition of photochemical reduction of nitroblue tetrazolium (NBT), using the method of Beauchamp & Fridovich, (1971).

Sampling and chemical analysis of rhizosphere soil: Rhizosphere soil was collected from 7-10 cm below the soil surface at 57 DAS. The samples were sieved and processed for the isolation of rhizobacteria.

Soil physicochemical analysis: For the estimation of organic matter of soil method of Walkley and Black, (1934) was followed. Macro- and micronutrients of soil were determined by the method of Soltanpour & Schwab (1977). Accumulation of nutrient in treated leaves was determined by the method of Piper *et al*., (1947). Leaf P was estimated by the method of Jackson (1973), while NO_3-N was determined by the method of Cataldo *et al*., (1975). Indoleacetic acid (IAA) and gibberellic acid (GA_3) were extracted and determined by the method of (Kettner & Doerffling, 1995).

Benefit cost ratio (BCR): Calculations for benefit cost ratio per hectare were made according to the formula given by Mehmood *et al*., (2011).

$$\text{BCR} = \frac{\text{Value of gross production} - \text{Cost of inputs (investments)}}{\text{Cost of inputs (investments)}}$$

Statistical analysis

Completely Randomized Design (CRD) and Randomized Complete Block Design (RCBD) were followed for pots and field experiment respectively. Data were analysed by Statistix software version 8.1 for Analysis of Variance (ANOVA). Mean values were compared according to Steel & Torrie, (1980) by least significant difference (LSD) at p = 0.05.

Results

Bacterial strains Cc-1, Cc-2 and Cc-3 were cultured on LB at 37°C for 24 hr. and identifed phenotypically and genotypically. The colonies of Cc-1 and Cc-2 were round white, while coloies for Cc-3 were round yellow. All the colonies ranged 2.5-4.5 µm in size. Only Cc-1 strain was gram positive with spore production ability. All three strains were positive for urease, oxidase, catalase and for utilization of citrate, fructose and mannose. For characterization of strains, 16S *rRNA* genes were amplified and sequenced. Amplified PCR products were sent to Microgen Korea for sequencing. Strain Cc-1 clustered closely with *B. cereus*, Cc-2 with *P. moraviensis* and Cc-3 with *S. maltophilia.* The rRNA gene' s nucleotide sequences of strains were submitted to GenBank with accession numbers LN714048 for *B. cereus*, LN714047 for *P. moraviensis* and LN714049 for *S. maltophilia.*

All three bacterial strains existing in *C. ciliaris* root powder were recovered from sterilized soil of pots, grown plants after 57 d and 122 d of inoculation (Fig. 1). *B. cereus* (Cc-1) exhibited 25% higher survival than *P. moraviensis* at 57d and 122d, post application of root powder. Lowest survival efficiency was recorded for *S. maltophilia.*

The soil organic matter receiving sterilized root powder treatment (RP) was significantly higher (32% and 58%) in field and pots grown plants, over control (Table 1). Application of root powder in un-sterilized form (RP + PGPB), enhanced the organic matter of soil by 44% and 45% in pots and field grown plants, respectively (n = 4, p = 0.05).

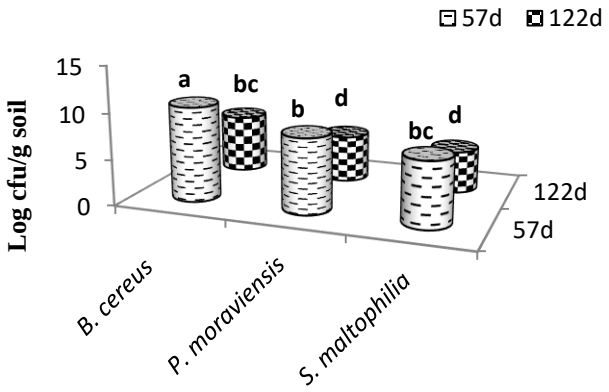

Fig. 1. Colony forming unit (cfu g^{-1} soil) of naturally occurring PGPB in root powder of *Cenchrus ciliaris*. Measurements were made from soil samples of pots grown plants, collected after 57d and 122d of root powder application.

Though no significant effects were observed over control in NO_3-N contents of rhizosphere soil of field (Table 1), but 36% higher NO_3-N were recorded in root powder + PGPB treatment. The P content was increased by 24% and 60% in pots and field grown plants, receiving root powder and PGPB treatment. Root powder in sterilized form improved the P content of rhizosphere soil of pots grown plants by 30%. K content of rhizosphere soil was 28% higher in field grown plants when root powder was applied with PGPB. Increase in Mg content of the soil was 35% in field and 67% in pots over control, receiving root powder application with PGPB.

The accumulation of NO_3-N in leaves was 31% and 39% higher in pots and field grown plants, respectively (Table 2). The accumulation of P content were 29% higher in root powder treatment, which was further improved by 12% when root powder was applied with PGPB. P contents were 32% higher in field and 25% in pots grown plants. Root powder application enhanced the K and Ca accumulation in leaves by 35% in pots grown plants while the Mg content was increased by 64%. The accumulation of K, Ca and Mg were improved by 35-40% in field grown plants, when root powder was applied with PGPB.

The increase in plant height was 28% over control at both stages in pots grown plants (Table 3). The root powder treatment promoted plant growth by increasing plant height in field grown plants. The increase in plant height was 30% over control in combine application of root powder with PGPB.

In pots grown plants an increase in fresh weight was 29% at early vegetative stage, following the root powder application with PGPB (Table 3). In field grown plants, increase in fresh weight (g) of aerial parts was 21% higher over control, receiving the root powder treatment (Table 4). Root powder with PGPB enhanced the fresh weight of plant by 33% at early and late vegetative stages. The increase in number of plant/m^2 was 16-30% in field grown plants in RP+ PGPB treatment.

Notable increase (21%) was observed in Chlorophyll contents of potted plants at early vegetative stage RP + PGPB treatment (Table 4). This increase was 25% at late vegetative stage.

In pots grown plants protein contents (mg g^{-1}) were 40% higher over control at early and late vegetative stage receiving root powder treatment ($p = 0.05$). This increase was 100% at both stages when root powder was added with PGPB (Table 3). In field grown plants, 50% greater protein content was observed over control at both the stages (Table 4).

At 57 d of sowing, sugar contents of pots and field grown plants were 16-20% higher in over control plants treated with root powder. Sugar and proline contents were 35% greater in field grown plants, only at early vegetative stage (Table 3). Decreases in sugar and proline were greater at late vegetative stage in field and pots grown plants.

The increase in antioxidant enzyme superoxide dismutase (SOD) activity (Fig. 2), was 30% over control at early and late vegetative stage of pots grown plants, following the root powder treatment ($p = 0.05$). Root powder addition with PGPB increased SOD activities by 86% and 45% at early and late vegetative stages. In the field grown plants receiving root powder treatment, SOD activity was 25% and 21% higher at early and late vegetative stage, respectively. PGPB addition with root powder further improved 32% and 26% SOD at early and late vegetative stage.

The increase in POD activity of pots and field grown plants at early vegetative stages were 20% over control following the root powder treatment (Fig. 2). The root powder addition with PGPB increased POD activity by 52% and 35%. The increases in POD activity at early vegetative stage were 47% and 37% over control, in field grown plants following the root powder application. These increases were 60% and 49% when root powder was added with PGPB.

In pot grown plant, root powder treatment increased indole acetic acid (IAA) by 35% and 38% over control at early and late vegetative stage respectively (Fig. 3). Root powder with PGPB exhibited 74% and 50% increases in IAA contents at early and late vegetative stage respectively ($p = 0.05$). The increase in IAA contents of field grown plants at early and late vegetative stages were 30-35% over control, following the root powder treatment. Root powder addition with PGPB increased IAA contents by 35% and 57%.

At early vegetative stage, GA contents of leaves receiving root powder treatment was increased by 47% over control, in potted and field grown plants (Fig. 3). At late vegetative the increase in GA contents was 28% in root powder treatment. The root powder with PGPB increased GA contents by 65% and 33%. The increase in GA contents at early vegetative stage was (67-75%) over control in field grown plants, following the root powder application with PGPB.

The root powder addition in un-sterilized form improved seeds/ spike by 20% over control ($p = 0.05$). The increase in spike length, seeds/spike (Table 5) was 26% over control in pots grown plants, receiving root powder treatment with PGPB. The increase in spike length, seeds/spike was 30% over control in field grown plants. Increase in seeds/ spike was 28% in potted plants over control.

The cost economic benefit ratio for the production of per hectare wheat grown in field was 1.19. The results indicate that *Cenchrus ciliaris* application of root powder of *C. ciliaris* may increase the farmer's benefits by 39%.

Table 1. Concentrations of chemical elements in soil (g Kg^{-1}) of wheat in response to root powder applications at 57 DAS in pot and field experiment.

Treatment	Field experiment			Pot experiment			LSD
	C	RP	RP+ PGB	C	RP	RP+ PGB	
O.M (%)	0.57c ± 0.08	0.77b ± 0.07	0.81b ± 0.05	0.45d ± 0.04	0.71b ± 0.08	0.83a ± 0.06	1.68
NO_3-N	20.43b ± 0.44	21.11b ± 0.21	24.24a ± 0.36	14.42c ± 0.23	16.15c ± 0.41	19.52b ± 0.6	2.41
P	4.71b ± 0.2	4.88b ± 0.11	5.82a ± 0.19	1.45e ± 0.01	1.88d ± 0.09	2.32c ± 0.16	3.92
K	77.41b ± 2.9	84.72b ± 2.31	92.22ab ± 1.88	92.21ab ± 2.23	102.23ab ± 2.43	118.11a ± 1.18	4.45
Ca	27.5ab ± 0.76	28.12ab ± 0.7	31.17a ± 0.11	32.89a ± 0.81	33a ± 0.66	35.76a ± 0.79	2.87
Mg	4.56c ± 0.21	4.9c ± 0.16	6.05b ± 0.18	6.66b ± 0.89	7.88b ± 0.14	11.15a ± 0.64	4.01

RP = Soil treated with sterilized root powder, RP + PGB = Soil treated with unsterilized root powder with plant growth promoting bacteria, C = Untreated control. Values are mean of four replicates. Values mention with different alphabets are significantly different (p = 0.05)

Table 2. Concentrations of chemical elements in leaves (g Kg^{-1}) of wheat in response to root powder applications at 57 DAS in pot and field experiment.

Treatment	Field experiment			Pot experiment			LSD
	C	RP	RP+ PGB	C	RP	RP+ PGB	
NO_3-N	2.21b ± 0.11	2.44bc ± 0.1	2.89a ± 0.1	1.91c ± 0.13	2.1b ± 0.09	2.66a ± 0.08	3.33
P	2.24c ± 0.15	2.88b ± 0.11	3.19a ± 0.22	1.81d ± 0.14	2d ± 0.13	2.74b ± 0.15	4.22
K	9.38bc ± 0.32	10.31b ± 0.21	13.29a ± 0.34	9.11bc ± 0.66	9.74bc ± 0.4	12.26a ± 0.65	3.13
Ca	4.46c ± 0.17	4.68c ± 0.11	5.88a ± 0.12	3.82d ± 0.18	3.98d ± 0.19	5.34b ± 0.23	2.12
Mg	1.59c ± 0.04	1.9b ± 0.04	2.14a ± 0.03	1.25d ± 0.08	1.44cd ± 0.04	1.89b ± 0.03	4.12

RP = Soil treated with sterilized root powder, RP + PGB = Soil treated with unsterilized root powder with plant growth promoting bacteria, C = Untreated control. Values are mean of four replicates. Values mention with different alphabets are significantly different (p = 0.05)

Table 3. Effects on growth, physiological and yield parameters of wheat in response to root powder application in a pot experiment. Plants were sampled at early vegetative stage (57 DAS), late vegetative stage (122 DAS).

Treatment	Pot experiment						LSD
	E.V			L.V			
	C	RP	RP+ PGB	C	RP	RP+ PGB	
Plant height (cm)	24.5d ± 1.05	26.5d ± 1.1	31.5c ± 1.11	38.25b ± 2.85	41.5b ± 1.08	49a ± 2.77	7.21
Fresh weight (g)	1.07d ± 0.09	1.14c ± 0.07	1.36c ± 0.07	3.8b ± 0.17	4.12ab ± 0.08	4.48a ± 0.33	4.6
Chlorophyll (nmol chl/ cm^2)	35.38ab ± 1.95	36.5ab ± 1.43	42.88a ± 2.59	30.5c ± 2.41	32.75c ± 1.87	38.5ab ± 2.18	5.22
Protein (mg g^{-1})	64.75c ± 3.75	90.5b ± 2.91	130.03a ± 5.63	60.55c ± 4.75	87.5b ± 3.95	115.31a ± 3.43	6.28
Sugar (mg g^{-1})	135.5c ± 3.75	161b ± 2.34	188.75a ± 2.22	83.12e ± 3.03	101.5cd ± 2.52	118.23cd ± 2.5	4.3
Proline (µg g^{-1})	153.75b ± 4.05	165.5ab ± 2.95	181.5a ± 3.22	77.5d ± 2.75	92.5cd ± 3.95	110c ± 1.62	4.52

P = Soil treated with sterilized root powder, RP + PGPB = Soil treated with unsterilized root powder with plant growth promoting bacteria, C = Untreated control. E.V = Early vegetative stage, L.V = Late vegetative stage. Values are mean of four replicates. Values mention with different alphabets are significantly different (p = 0.05)

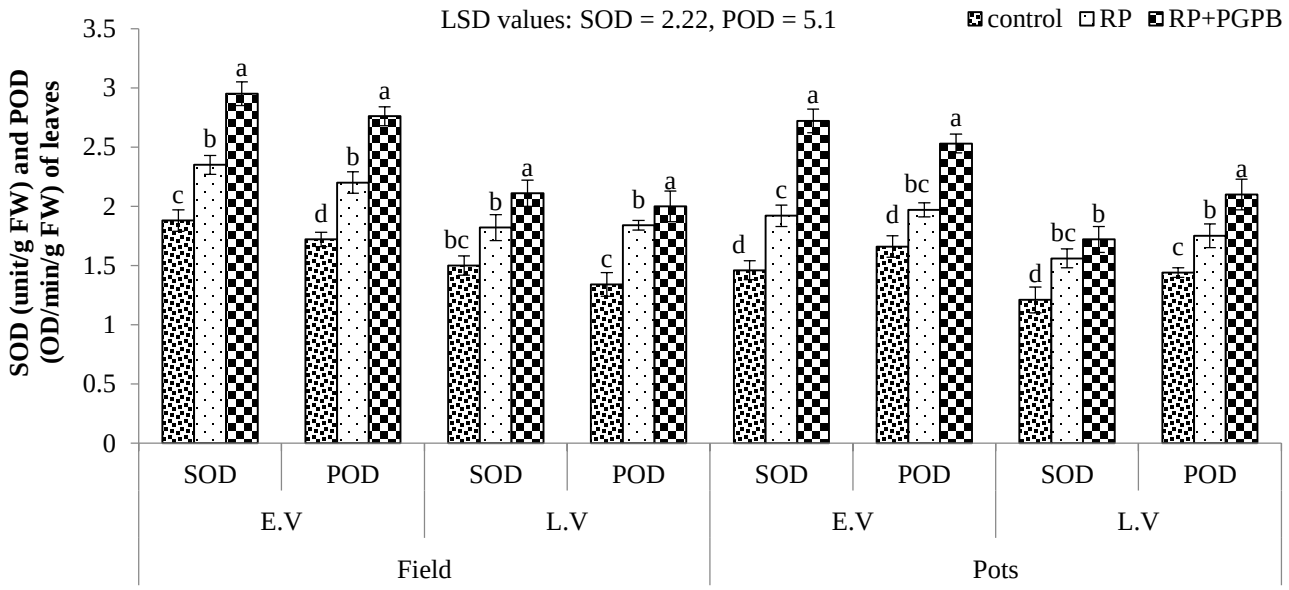

Fig. 2. Superoxide dismutase (SOD) and peroxidase (POD) activities of leaves. Results are mean of four replicates with ± SE bar. Values represented over the bars with different alphabet reflects significantly differences at (p = 0.05). RP = Soil treated with sterilized root powder, RP + PGPB = Soil treated with unsterilized root powder with plant growth promoting bacteria, C = untreated control. E.V = early vegetative stage, L.V = late vegetative stage.

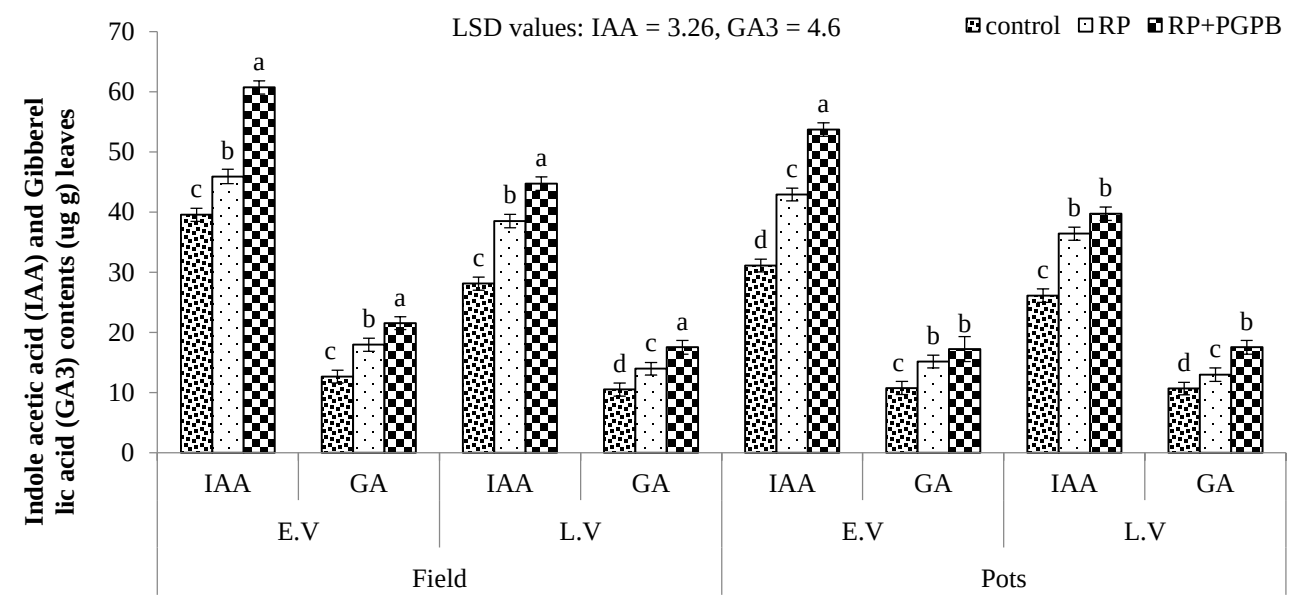

Fig. 3. Indole acetic acid (IAA) and gibberellic acid (GA_3) contents of leaves. . Results are mean of four replicates with ± SE bar. Values represented over the bars with different alphabet reflects significantly differences at (p = 0.05). RP = Soil treated with sterilized root powder, RP + PGPB = Soil treated with unsterilized root powder with plant growth promoting bacteria, C = untreated control. E.V = early vegetative stage, L.V = late vegetative stage.

Discussion

The cfu of *P. moraviensis* and *B. cereus* were higher in root powder as compared to *Stenotrophomonas maltophilia.* Inoculation of *P. moraviensis* and *B. cereus* isolated from *Cenchrus ciliaris* rhizosphere and applied on wheat had higher cfu than application of these PGPB in root powder form (Hassan & Bano, 2014), convincingly, this is due to culture media.

Application of root powder with or without associated PGPB (*B. cereus*, *P. moraviensis* and *S. maltophilia*) increased soil organic matter of pots and field grown plants. Organic matter and carbon content in soil are the indicators of biological activities and richness of microflora (Ghosh *et al*., 2003). The presence of higher organic matter in sterilized or un-sterilized root powder possibly increased the organic matter of treated soil and improved PGPB survival. Prakash *et al*., (2007) determined the efficacy of FYM (Farm Yard Manure) and found it as a good source of organic matter. Similarly, use of maize straw, sugarcane husk and rice husk also increased organic matter of soil and assisted PGPR survival (Ogbo & Odo, 2011; Hassan & Bano, 2015).

Table 4. Effects on growth, physiological and yield parameters of wheat in response to root powder application in field experiment. Plants were sampled at early vegetative stage (57 DAS), late vegetative stage (122 DAS).

Treatment	Field experiment						LSD
	E.V			L.V			
	C	RP	RP + PGB	C	RP	RP + PGB	
Plant height (cm)	29.25d ± 1.75	31.5d ± 1.11	37.25c ± 2.35	46.25b ± 1.88	48b ± 1.15	56.75a ± 1.62	5.12
Fresh weight (g)	2.21d ± 0.03	2.66c ± 0.05	2.94c ± 0.13	5b ± 0.07	5.5b ± 0.07	6.89a ± 0.11	3.16
Chlorophyll (nmol chl/ cm^2)	42.9a ± 1.45	44a ± 1.7	47.93a ± 2.13	37.92b ± 2.22	39.5b ± 1.49	44.53a ± 2.35	5.09
Protein (mg g^{-1})	102.09c ± 3.99	111.5bc ± 1.75	155.31a ± 8.11	86.5d ± 2.87	94.5cd ± 1.75	126.51b ± 7.01	4.86
Sugar (mg g^{-1})	173bc ± 4.03	201.5ab ± 3.39	233.75a ± 1.19	149.5cd ± 2.49	166bc ± 3.03	188b ± 2.41	6.27
Proline (µg g^{-1})	173.75bc ± 2.5	198.56bc ± 3.03	241.5a ± 3.13	149.5d ± 2.12	172.5bc ± 4.03	211a ± 4.5	5.31

P = Soil treated with sterilized root powder, RP + PGB = Soil treated with unsterilized root powder with plant growth promoting bacteria, C = Untreated control. E.V = Early vegetative stage, L.V = Late vegetative stage. Values are mean of four replicates. Values mention with different alphabets are significantly different (p = 0.05)

Table 5. Effects on yield parameters of wheat in response to root powder application in pot or field experiment. Plants were sampled at maturity (159 DAS).

Treatment	Pots experiment			Field experiment			LSD
	C	RP	RP + PGB	C	RP	RP + PGB	
Spike length (cm)	6.6d ± 0.3	6.77d ± 0.2	8.33c ± 0.23	9b ± 0.18	9.5b ± 0.34	11.7a ± 0.12	2.23
seeds /spike	44b ± 2	50.5ab ± 2.12	56.5a ± 0.5	52b ± 0.75	62.5a ± 2.12	67.75a ± 2.1	5.11
seed weight (g)	29.18b ± 0.48	30.33b ± 0.26	31.26b ± 0.32	46.45a ± 0.16	47.76a ± 0.38	50.51a ± 0.34	2.9

P = Soil treated with sterilized root powder, RP + PGB = Soil treated with unsterilized root powder with plant growth promoting bacteria, C = Untreated control. Values are mean of four replicates. Values mention with different alphabets are significantly different (p = 0.05)

Soil health and fertility is determined by the presence of macro and micro nutrients (Cakmakci *et al*., 2007). NO_3-N and P-contents of soil and leaves were increased significantly by the application of root powder in sterilized or unsterilized form. Increase in P and NO_3-N content may be attributed to the nitrogen fixing and P-solublization ability of PGPR, which was augmented by the presence of organic matter and C- sources (Orhan *et al*., 2006; Aslantas *et al*., 2007; Elkoca *et al*., 2008).

Nutrients (K, Ca and Mg) increase in rhizosphere soil and accumulation in leaves of root powder treated plants might be ascribed to PGPB (*B. cereus*, *P. moraviensis* and *S. maltophilia*), potential in abetting soil health improvement and nutrients translocation (Rana *et al*., 2012). Sheng (2005) reported that availability and accumulation of K, Ca and Mg in response to the application of PGPB positively affected plant growth. Similarly, greater accumulation of nutrients was observed in apple leaves following the application of PGPB (Karakurat & Aslantas, 2010).

Nitrogen and Mg are integral parts of chlorophyll structure and increase in chlorophyll is correlated with improved N and Mg acquisition in leaves treated with PGPB (Vivas *et al*., 2003). Decrease in protein and soluble sugar in older leaves (at late vegetative stage) is associated chlorophyll reduction and leaf senescence (Panda *et al*., 2013).

Soluble sugar and protein protect macro-molecules from degradation which is mediated by osmotic adjustment (Udawat *et al*., 2016). Older leaves accumulated less protein contents at late vegetative stage which insinuated the decreased level of pyrroline-5-carboxylate reductase activity. pyrroline-5-carboxylate reductase enzyme is key for proline metabolism and it is degraded in aged plants (Claussen, 2005). The evidenced increase in soluble sugar and protein in present research is in agreement with previous findings where these constituents were improved by PGPB inoculation (Parida *et al*., 2002; Younesi & Moradi, 2014).

Antioxidant enzymatic system is essential for detoxification of ROS (Reactive Oxygen Species) (Sharma *et al*., 2012). During present study, antioxidant activities were significantly higher in plants treated with root powder and PGPB. Our results are in harmony with previous observations made on wheat leaves (Upadhyay *et al*., 2012). The activities of antioxidants were lower at late vegetative stage in wheat leaves. In mature leaves change respiratory rate effect energy metabolism of plant which result in physiological changes (Prochazkova *et al*., 2001).

Phytohormone production is the peculiarity of Plant growth promoting bacteria and different bacteria have different potential of phytohormone production (Mirza *et al*., 2001). It has been reported that endophytic PGPB are more capable of producing phytohormones than PGPR (Coutinho *et al*., 2015). Three endophytes (*Bacillus cereus*, *Pseudomonas moraviensis* and *Stenotrophomonas maltophilia*) found in root powder have been documented as phytohormones producers (Naz & Bano, 2012; Kloepper

et al., 2013; Sivasankari *et al*., 2014). Higher accumulation of IAA, GA and ABA following the application of un-sterilized root powder suggest that PGPB were involved in improving phytohormones contents.

In present study some yield attributes of wheat were improved significantly in the presence of PGPB+ RP. These results indicate that root powder treatment increased water and nutrients uptake and photosynthesis rate in plants (Baset-Mia *et al*., 2010). Previously, application of *P. moraviensis* as bio-inoculant was found as beneficial source for improving banana growth and production (Ngamau *et al*., 2012).

Conclusions

The root powder of *C. ciliaris* in sterilized or un-sterilized form is rich with organic matter and it harbours the PGPB that are valuable for agriculture. Efficacy of root powder in un-sterilized form with PGPB has potential of enhancing nutrients in soil and translocation in aerial parts. Application of root powder in un-sterilized form (with PGPB) imparts positive effects on soil health and improves growth, physiology and yield of wheat. Root powder being a rich source of carbohydrates and protein supported survival of PGPB and it may be recommended as carrier for biofertilizer industry.

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