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EFFECTS OF NOVEL CARRIER-BASED FORMULATIONS ON WHEAT GROWTH, PHYSIOLOGY AND YIELD

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Abstract

Sugarcane husk hold rich carbon sources and can be used as carrier for biofertilzer industry. Two formulations of carrier-based biofertilizers were made comprising consortium of plant growth promoting rhizobacteria (PGPR) *Pseudomonas moraviensis* and *Bacillus cereus.* Formulation-I comprised the consortium of *Bacillus cereus* and *Pseudomonas moraviensis* with maize straws powder and Formulation-II comprised same consortium and sugarcane husk as carrier material. *Pseudomonas moraviensis* and *Bacillus cereus* were isolated from halophytic herb *Cenchrus ciliaris* (L) and maintained greater colony forming unit (cfu) in the carriers after 40 d of incubation. Phosphate solubilization potential and antifungal activities of PGPR was remarkable and used as bio-inoculant on wheat, grown in saline sodic soil of field at Soil Salinity Research Institute, Pindibhattian. Both formulations alleviated the adverse effects of salt stress by decreasing electrical conductivity (EC), sodium absorption ratio (SAR), regulating ion homeostasis by decreasing Na and Cl content, and improving K, P, N-NO_3 and organic matter by 20- 40% in the wheat rhizosphere. There were 20% decreases in Na and 30-45% increase in K in wheat leaves over control. Formulations and consortium treatments increased sugar, proline and phytohormones gibberellic acid (GA), indole acetic acid (IAA) and abscisic acid (ABA) content of leaves by 50-90 % over control plants. Antioxidants enzymes activities were significantly higher in consortium and formulation treatments. From the results, it was concluded that Formulation- B comprising sugarcane husk as carrier was more effective than formulation having maize straw as carrier.

Key words: *Bacillus cereus*; Biological waste application; *Pseudomonas moraviensis*; Saline sodicity; Bio-formulations.

Introduction

The bio-fertilizers are important sources of nutrient recuperation and plant growth promotion consequently provokes agricultural sustainability. The bio-fertilizers are recommended for better nitrogen fixation, Nutrients acquisition, P- solubilization and yield improvement in cereals (Gao *et al.*, 2020).

Different carriers have been used to protect the viable cells of PGPR against biotic and abiotic stresses (Lobo *et al*., 2019). The biodiversity and activities of bacteria are associated with the quality and properties of carriers. A suitable carrier is a prerequisite for developing a commercial level bio- fertilizer because it increased shelf life and protect PGPR endurance against adverse environmental conditions. Peat, lignite, charcoal, rice husk, humus rich soil and other carriers have been tested as carrier (Thiyageshwari *et al*., 2018).

Every year salt affected land is rigorously increasing resulting heavy economic losses (Shahid *et al*., 2020). In Pakistan salinity problem is very serious and it captures about 4.5 Mha located in different provinces (Aslam, 2016).

Graminaceous crops are severely affected by salinity and modern agriculture uses several agricultural practices to alleviate salinity. Inoculum based and carrier based bio-fertilizers are the modern tools for salinity alleviation. Extensive use of synthetic fertilizers have instigated pollution, depleted nutrient reservoirs, and destroyed beneficial microflora and insects. Application of bio-fertilizer is productive, economical, and eco-friendly for sustainable agriculture practices (Gao *et al*., 2020).

Bacterial species belonging to different genera and most importantly *Bacillus* and *Pseudomonas* are considered as good inoculants for plants, forests and cereals (Egamberdieva *et al*., 2019). Consortia of *Bacillus* and *Pseudomonas* with root colonizers are potential sources of wheat yield and improvement of soil fertility (Menéndez & Paço, 2020).

Bio-fertilizers have been formulated to explore native and exotic PGPR under normal growing conditions in the field, but no detailed studies have yet revealed the affectivity of carrier based bio-fertilizer under salt stressed areas. Halophytic bacteria are well adopted to salt stressed habitat and may induce tolerance to high salinity in their host plants (Meinzer *et al*., 2023).

To date, several environmental friendly PGPR strain of *B. cereus* like YL6, SA1, MEN8, ALT1, ERBP, GGBSTD1, AK1, T4S, WSE01, AR156 and C1L have been recorded by different researchers. This bacterium has tendency to perform actively in growth chamber, greenhouse, wire house and field because of phosphate solubilization, indole-3-acetic acid (IAA) and aminocyclopropane-1-carboxylic acid (ACC) deaminase and affecting plant growth by nutrient uptake production content (N, P, and K), antioxidant enzymes and osmolytes productions under salt stress or (Kulkova *et al*., 2023*). Pseudomonas moraviensis* as PGPR (P solubliser and IAA producer), isolated from fluvo-aquic soils has been reported to enhance wheat yield by 14% (Wang *et al.*, 2022).

Bacteria found in rhizosphere of halophytic plants have ability to perform better under stress and in the presence of C-sources. Provision of C-sources in the form

of sugarcane husk or maize straw powder may enable plants to perform better in saline sodic field conditions and improve wheat growth and physiology.

Present work was aimed to evaluate the positive role of two bio-fertilizer formulations prepared by the composition of two halophytic bacteria *Bacillus cereus* and *Pseudomonas moraviensis* with two different carriers. A comparative study was made to find the promoting effects of consortium and formulations on wheat under saline sodic field condition, using PGPR singly or in consortium with two different carriers.

Material and Methods

Soil preparation and sowing: Prior to sowing, plots sizes (6 m^2) were made and a distance of 25 cm was maintained between two rows. Treatments comprised of the consortium of *Bacillus cereus* and *Pseudomonas moraviensis*, Formulation-I (consortium + maize straw powder), Formulation-II (consortium + sugarcane husk) and un-inoculated control (C) contained no carrier and PGPR. Four replicates were made for each treatment and randomized complete block design (RCBD) was followed during sowing. Seeds coated with consortium and bio-formulations were sown by the conventional hand drill method.

PGPR based inoculum preparation: *Pseudomonas moraviensis* and *Bacillus cereus* having accession No. LN714047 and LN714048 respectively were used in bio-fertilizer Formulation-I and application on wheat, in a saline sodic field for two years trial. *Pseudomonas moraviensis* and *Bacillus cereus* were cultured on Luria–Bertani (L.B) culture media for 2d.

Formulation of bio-fertilizer: Sugarcane husk was obtained from juice hawkers and maize straw was collected from cropping field. Both were shade dried before milling into fine powder (Anex grinder KC106). Powder was sieved by Sieve (ANTAI China pore size 0.20-0.31 mm) and autoclaved twice. In each 50 g of powder L.B broth culture of consortium (25 ml) was added. In a laminar flow (sterile conditions), each formulation was incubated for 24 h and packed in UV sterilized polythene bags.

At 25 d of packaging, Formulation-I and Formulation-II had $13x10^8$ CFU g^{-1} for *P. moraviensis* and $19x10^8$ CFU g^{-1} for *B. cereus* (Fig. 1).

Inoculation studies: Wheat cv. Inqlab-91 was used for experiments in both years. Field experiment was conducted at Soil Salinity Research Institute, Pindibhatian, Pakistan. Wheat seeds were sterilized with 70% ethanol for 3 min and shaken with 10% chlorox for 5 min. Seeds were coated with each bio-formulation in a way that each 250 g seeds were treated with 2 g of formulation. After shade drying of 20 min seeds were sown. Average temperature of the cropping area was $25^0 \pm 2^0$C, 13 h dark period and a photo period of 11 h.

Phosphate solubilization and antifungal activity of PGPR: Bacterial isolates were checked for phosphate solubilizing character on Pikovskaya's agar medium media as expressed by Vyas *et al*., 2007. Bacterial cultures were spotted in the middle of the plate by wire loop and incubated at 30°C for 7 days. Appearance of clear zone around spot determined Phosphorus solubilization activity of bacteria. Solubilization strength of bacteria was determined by formula:

$$\text{Solubilization index} = \frac{\text{Colony diameter + Halo zone diameter}}{\text{Colony diameter}}$$

Physicochemical analysis of soil: Soil was sampled from the wheat roots at the depth of 7-10 cm after 57 days of sowing. The pH of rhizospheric soil was determined by the method of McKeague (1978) and Mclean (1982). Soil Nitrate-N (NO_3-N) and phosphorus (P) were obtained by Reitemeier (1943) method. For the determination of organic matter in soil samples Walkley & Black, (1934) method was applied.

Sugar estimation: Soluble sugar in the leaves was determined by Dubois *et al*., (1956), which was modified by Johnson *et al*., (1966). Plant tissues were homogenized in 10 ml of distilled water and centrifuged at 3000 × g for 5 min and supernatant was collected. Sulphuric acid (5 ml) was in 0.1 ml of supernatant and after 4 h absorbance was recorded at 420 nm.

Protein content of leaves: The protein content of leaves was determined by the method of Lowry *et al*., (1951). Fresh leaves (0.1 g) were mixed in phosphate buffer (pH 7.5) and ground for 2-5 min in a mortar. Mixture was centrifuged for 10 min at 1000 × g. Supernatant (0.1 ml) was separated and diluted with water to make final volume of 1 ml. After treating with Folin Phenol reagent, and incubation for 30 min, absorbance was recorded at 650 nm.

Proline estimation and antioxidant assays: Free proline content of leaves was measured by the method of Bates *et al*., (1973). Plant material (0.5 g) was homogenized in 10 ml of 3% aqueous sulphosalicylic acid. Filtrate (2 ml) was treated with 2 ml acid ninhydrin and 2 ml of glacial acetic acid in a test tube for 1 h at 100°C. The reaction mixture was extracted with 4 ml toluene and stirred for 15-20 Sec. The absorbance of toluene layer was read at 520 nm against toluene as blank. Extraction of antioxidant enzymes superoxide dismutase (SOD) and peroxidase (POD) was made following the method of Vetter *et al*., (1958) and determined by Beauchamp & Fridovich, (1971) using inhibition of photochemical reduction of nitroblue tetrazolium (NBT).

Determination of phytohormones: Three phytohormones (IAA, GA and ABA) were extracted and purified by the method of (Kettner & Doerffling, 1995). Plant tissues (1 g) were blended with butylated hydroxyl toluene (BHT) and methanol (80%) at a temperature of 4°C. The mixture extracted for 72 h and solvent was changed after every 24 h. Tissue extract obtained was centrifuged to collect supernatant. Rotary thin film evaporator (RFE) was used to reduce supernatant into aqueous phase and pH was adjusted to 2.5-3.0. Ethyl acetate was used for partitioning which was later dried by RFE. Dried material was re-dissolved in 1 ml of 100% methanol. Samples were

analyzed on HPLC (Shimadzu, C-R4A Chromatopac; SCL-6B system controller) equipped with UV detector and C-18 column (39 x 300 mm) for identification of phytohormones. The detection of IAA was made at 280 nm and ABA, GA_3 were detected at 254 nm.

Statistical analyses

Field experiment was designed according to Randomized Complete Block Design (RCBD). The data obtained from field experiment of two consecutive years (2020 and 2021) was subjected to statistical analyses and Analysis of Variance (ANOVA) by *Statistix* 8.1 version. For each treatment, data of five replicates were acquired and mean values of two years were parted by the method of Steel & Torrie (1980) using least significant difference (LSD) at p = 0.05 and Standard Error (SE).

Results

Survival of PGPR in bio-formulations: The colony forming Unit (CFU) of both PGPR *Pseudomonas moraviensis* and *Bacillus cereus* in carriers was increased linearly with incubation period. At 40d CFU of *Bacillus cereus* was 60% higher in sugarcane husk compared to *Pseudomonas moraviensis*. The CFU of both PGPR was 10-15% higher in sugarcane husk as compare to maize straw (Fig. 1).

Effect of carrier based formulations on soil nutrients: The consortium of PGPR decreased electrical conductivity of soil by 15% over control. Formulation-I and Formulation-II decreased EC by 21% (Table 1). Decreases in pH in consortium and formulations treatments were 7-8% over control. Sodium absorption ratio (SAR) was decreased by 28% in consortium treatment while Formulation-I and Formulation-II decreased SAR by 36% and 31% respectively. Performance of formulations was better (11% greater decrease) compared to consortium treatments. Soil organic matter was increased significantly (27%) in consortium treatment, while 40% and 42% higher organic matter were found in Formulation-I and Formulation-II, respectively. Formulations increased organic matter (10%) than consortium treatment.

The consortium of PGPR exhibited 10% less Na^+ and Cl^- in soil while both the formulations decreased 18% Na^+ and 11% Cl^- over control. Increases in NO_3-N, P and K were 36%, 28% and 33% respectively in consortium treatments (Table 2). Formulation-II increased NO_3-N, P and K by 36%, 28% and 40% respectively over control. Increases in NO_3-N, P and K were 46%, 57% and 42% in Formulation-II treatments, respectively.

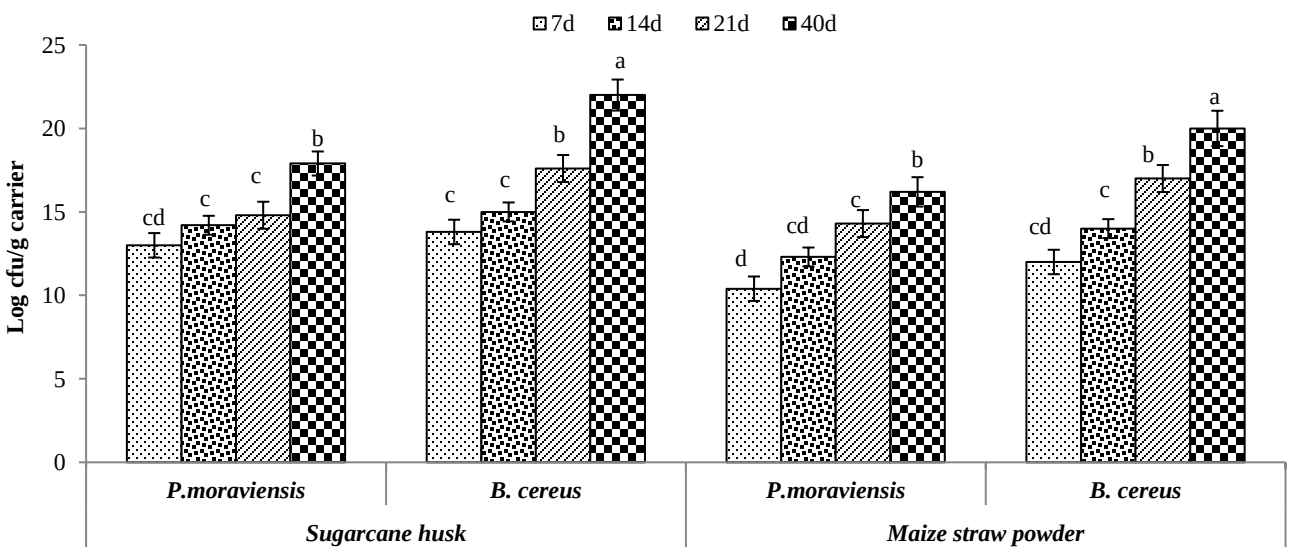

Fig. 1. Colony forming unit (CFU/g of carrier material) of *Pseudomonas moraviensis* and *Bacillus cereus* in co- inoculation after different intervals. Values given are mean of four replicates with ± standard error (SE). Values followed by different letters heading the bars are significantly different at p=0.05.

Table 1. Effects of bio-formulations on electrical conductivity (dS m^{-1}), pH, organic matter (%) and Sodium Absorption ratio (SAR) of rhizosphere soil of wheat after 57d of sowing (2-3 leaf stage). Values are mean of four replicates.

Treatments	Control*	Consortium	Formulation-I	Formulation-II
EC(dSm^{-1})	4.71 ± 0.15a	4.08 ± 0.14b	3.88 ± 0.12b	3.92 ± 0.18b
Soil pH	8.77 ± 0.12a	8.13 ± 0.12a	8.11 ± 0.15a	8.18 ± 0.17a
O. M (%)	0.85 ± 0.04d	1.09 ± 0.13ab	1.19 ± 0.05a	1.21 ± 0.11a
SAR	25.82 ± 0.13a	21.1 ± 0.23b	18.97 ± 0.17c	19.6 ± 0.24c

Consortium = *Pseudomonas moraviensis* + *Bacillus cereus*
Formulation-I = *Pseudomonas moraviensis* + *Bacillus cereus* + maize straw powder
Formulation-II = *Pseudomonas moraviensis* + *Bacillus cereus* + sugarcane husk
O.M = Organic matter. Control* = Untreated soil
Values followed by different letters in a column are significantly different (p=0.05)

Table 2. Effects of bio-formulations application on soil nutrients content of wheat (mg/kg) after 57 d of sowing (2-3 leaf stage). Values are mean of four replicates.

Treatments	Control*	Consortium	Formulation-I	Formulation-II
Na^+	121.09 ± 5.15a	109.13 ± 2.31b	101.29 ± 2.45b	103.12 ± 2.65b
Cl^-	198.06 ± 1.94a	181.32 ± 2.87b	177.11 ± 2.22c	174.12 ± 2.11c
NO_3-N	22.22 ± 0.22b	30.11 ± 0.43a	31.33 ± 0.39a	32.49 ± 0.12a
P	7.7 ± 0.09b	9.88 ± 0.45a	10.01 ± 0.54a	12.12 ± 0.22a
K^+	130.19 ± 7.45b	174 ± 3.34a	182.45 ± 2.16a	184.44 ± 2.03a

Consortium = *Pseudomonas moraviensis* + *Bacillus cereus*
Formulation-I = *Pseudomonas moraviensis* + *Bacillus cereus*+ maize straw powder
Formulation-II = *Pseudomonas moraviensis* + *Bacillus cereus*+ sugarcane husk
Control* = Untreated soil
Values followed by different letters in a column are significantly different (p=0.05)

Table 3. Effect of bio-formulations application on nutrients contents of wheat leaves (mg/kg) after 57 d of sowing (2-3 leaf stage). Values are mean of four replicates.

Treatments	Control*	Consortium	Formulation-I	Formulation-II
Na^+	16.7a (± 0.41)	13.33ab (± 0.31)	12.25b (± 0.24)	12.14b (± 0.51)
K^+	25.2c (± 0.31)	33.91b (± 0.08)	34.78b (± 0.11)	38a (± 0.62)
NO_3-N	2.42b (± 0.05)	3.3a (± 0.04)	3.56a (± 0.09)	3.68a (± 0.08)
P	3.27c (± 0.02)	4.52b (± 0.02)	5.66a (± 0.09)	5.83a (± 0.07)

Consortium = *Pseudomonas moraviensis* + *Bacillus cereus*
Formulation-I = *Pseudomonas moraviensis* + *Bacillus cereus*+ maize straw powder
Formulation-II = *Pseudomonas moraviensis* + *Bacillus cereus*+ sugarcane husk
Control* = Untreated soil
Values followed by different letters in a row are significantly different (p=0.05)

Table 4. Effects of bio-formulations on growth and yield parameters of wheat after 159 d of sowing (maturity stage). Measurements were made after 57 d of sowing (2-3 leaf stage). Values are mean of four replicates.

Treatments	Control*	Consortium	Formulation-I	Formulation-II
Height (cm)	30b (±0.5)	31.75b (±0.28)	35.5a (±0.34)	37a (±0.52)
Fresh weight (g)	1.66d (±0.01)	1.83c (±0.06)	1.97a (±0.04)	2.08b (±0.01)
Chlorophyll (µg/cm^2)	48.18b (±0.83)	55.2a (±1.45)	56.91a (±0.15)	57.28a (±0.68)
Protein (mg g^{-1})	78.28c (±2.86)	90.9b (±4.05)	103.39a (±6.61)	108.25a (±5.75)
Plant/m^2	216c (±5.5)	304.5bb (±5.59)	323.5a (±13.25)	329.75a (±6.25)
Spike length (cm)	7.15d (±0.05)	10.53c (±0.13)	11.1b (±0.17)	11.4a (±0.05)
Seeds/spike	35.5d (±0.5)	53.5c (±1.5)	59.5b (±1.81)	62a (±0.5)
Seed weight (g)	44.48b (±1.36)	51.14a (±4.11)	51.89a (±3.17)	52.84a (±1.98)

Consortium = *Pseudomonas moraviensis* + *Bacillus cereus*
Formulation-I = *Pseudomonas moraviensis* + *Bacillus cereus*+ maize straw powder
Formulation-II = *Pseudomonas moraviensis* + *Bacillus cereus*+ sugarcane husk
Control* = untreated soil
Values followed by different letters in a column are significantly different (p=0.05)

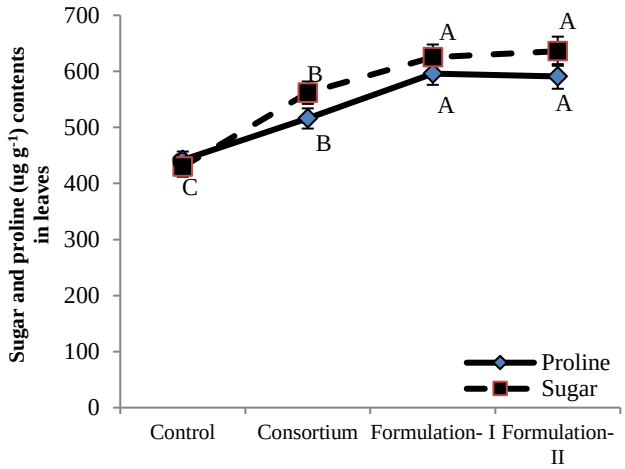

Fig. 2. Sugar and proline contents (ug g-1) of leaves at early vegetative stage of plant growth (after 57d of sowing). Values given are mean of four replicates ± SE. Values followed by different letters heading the bars are significantly different (p=0.05).

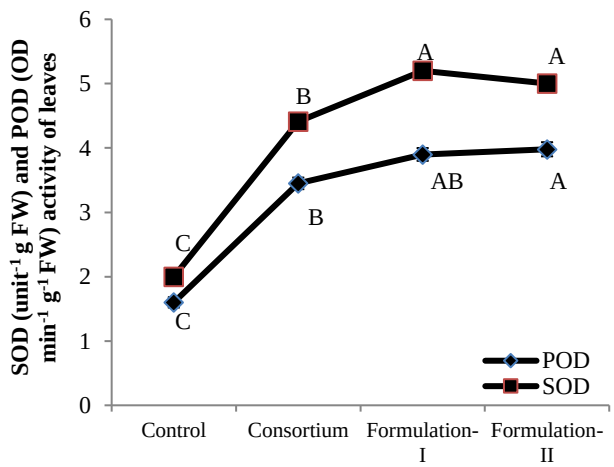

Fig. 3. SOD (unit^{-1g} FW) and POD (OD min^{-1} g^{-1} FW) activity of leaves at early vegetative stage of plant growth (57DAS). Values given are mean of four replicates ± SE. Values followed by different letters heading the bars are significantly different (P=0.05).

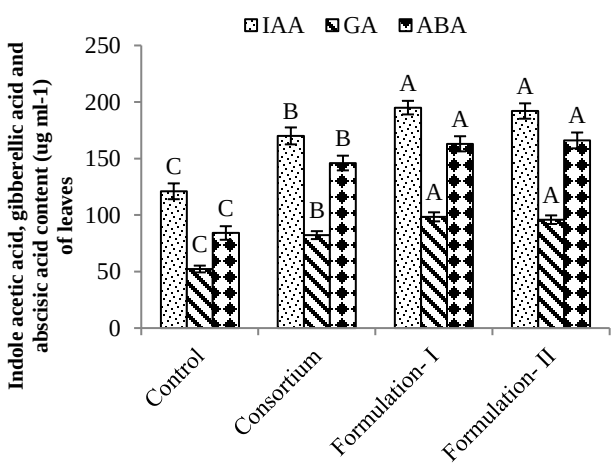

Fig. 4. Indole acetic acid, gibberellic acid and abscisic acid content (µg ml^{-1}) of leaves at early vegetative stage of plant growth (57DAS). Values given are mean of four replicates ± SE. Values followed by different letters heading the bars are significantly different (p=0.05).

Effects of carrier based formulations on nutrient in wheat leaves: Seeds inoculation with consortium decreased 25% Na^+ in wheat leaves compared to control (Table 3). Formulation-I decreased 36%, while Formulation-II decreased 38% Na^+ in leaves. Accumulations of NO_3-N, P and K in wheat leaves were 35-38% better in consortium than control. Formulation-I increased NO_3-N, P and K in leaves by 39%, 47% and 73% respectively, while these increments were 51%, 52% and 78% in Formulation-II treatment. Increase in P was 25% greater in formulations applications compared to consortium.

Effect of carrier based formulations on wheat growth and physiology: Plant height at 57 DAS was 12% higher than control in the consortium treatment. Formulation-I and Formulation-II increased plant height by 18% and 25%, respectively over control (Table 4). Notable (12%) increase was observed in formulations treatments compared to consortium. Fresh weight of plants at same stage was 10% greater in consortium treatment. Formulation-I improved fresh weight by 19% and Formulation-II by 24% over control.

Chlorophyll content in wheat leaves at 57 DAS was increased by 15% in consortium treatment, while both the formulations increased chlorophyll by 19% over control. Notable increase (14%) was observed in formulations treatments compared to consortium. Increases in protein content were 16% in consortium treatment while Formulation-I and Formulation-II exhibited 32% and 38% increases in protein content, respectively.

There was 16% higher proline content in wheat leaves treated with consortium and this increase was 33% in formulations treatments. Similarly soluble sugar was 29% higher in wheat leaves in consortium treatment and 43-45% higher sugar in formulations treatments (Fig. 2).

Peroxidase was improved by 87% in consortium treatment and Formulation-I and Formulation-II increased POD by 120% (Fig. 3). Similarly, SOD contents were 115% higher in consortium treatment. Formulation-I increased SOD by 143% and Formulation-II increased 150% SOD in wheat leaves over control. Formulation-I resulted 14% higher SOD over Formulation-II.

Phytohormone IAA accumulation was 43% higher in consortium application while formulations exhibited 58% higher IAA over control. There was 55% higher GA in leaves treated with consortium. Both formulations increased GA contents by 88-90% over control. Similarly, ABA in leaves treated with consortium was 78% higher over control and formulations application increased 97% ABA over control. All phytohormones (IAA, GA and ABA) were 13-20% higher in Formulation-I treatment compared to Formulation-II treatment (Fig. 4).

Effects of Bio-formulations on wheat yield: At 159 DAS, PGPR consortium significantly enhanced (40%) number of plants over control (Table 4). However, the contributory effects of both formulations were 52% higher over control. Spike length was 47% higher in consortium treatment. Formulation-I and Formulation-II increased 55% and 59% greater spike length over control, respectively. Plants treated with consortium had 51% greater seeds per spike over control. Formulation-I had 67% higher seeds per spike

and Formulation-II had 74% higher seeds per spike over control. The weight of 1000 seeds at 159 DAS in consortium and bio formulations treatment was 15% higher over control.

Discussion

Survival of PGPR with different carrier material is challenging and major hazard toward a sustainable bacterial formulation. The better survival of both PGPRs in the presence of carrier materials measure as colony forming unit indicates better survival of PGPR (Hussain *et al*., 2022). Carbon sources are very important for growth and survival of bacteria and carrier materials (sugarcane husk and maize straw) being C-sources enhance the CFU of the PGPR (Thiyageshwari *et al*., 2018). Additionally, P-solublization potential of both isolates increased the strength of Formulation-I and efficacy as PGPR. Strong P-solublization is correlated with members of *Bacillus* and *Pseudomonas* genera (Sultana *et al*., 2018).

Both the PGPR exhibited antifungal potential against different fungal cultures. Antifungal potential of bacteria make them compatible and enhance survival potential among indigenous microflora. Previously strong antifungal activity of *Bacillus* sp was detected against different fungal strains (Matevosyan *et al*., 2019). Similarly, *Pseudomonas fluorescens* exhibit strong antifungal potential (Shinde *et al*., 2019).

In present research, PGPR induced reclamation of salinity by decreasing salt ions Na^+, Cl^- and HCO^-_3 and increasing nutrients P and NO_3–N (Numan *et al*., 2018). Na^+ contents were declined enormously following the bio-inoculation of *Pseudomonas putida* and *Bradyrhizobium japonicum* under salinity stress (Egamberdieva *et al*., 2017). Single inoculum as well as carrier based formulation increased the organic matter in treated soil. Thiyageshwari *et al*., (2018) revealed that carrier based products being enriched with C-sources increased soil organic matter.

Co-inoculation was effective to decrees EC of the soil which indicates the synergistic relation between the two PGPR (Xie *et al*., 2018). The decline in sodium absorption ratio and electrical conductivity of saline sodic soil facilitates uptake of Ca^+, Mg^+, K^+, and P which in turn may be responsible for increased fresh weight and plant height (Alcívar *et al*., 2018). Accumulation of higher nutrients following the application of PGPR has been reported in maize (Pereira *et al*., 2020).

Carrier based formulation decreased Na^+, Na^+/K^+ and Na^+/Ca^+ uptake of leaves and enhanced K^+ accumulation which were important cues of plants under salt stress (Almeida *et al*., 2017). Similarly, improvement in nutrients uptake and mobilization of P, Ca and K ascribed the seeds establishment, thereby substantial increase in yield attributes (Pereira *et al*., 2020). The production and modulation of phytohormones particularly IAA and GA increased the proliferation of root system might account for the increased yield (seed number and seed size) (Ahmad *et al*., 2020).

Accumulation of higher proline and sugar contents in the inoculated plants manifests better osmoregulation that helps plants to withstand harsh environments (Ilangumaran & Smith, 2017).Müller *et al*., (2019) reported that *Azospirillum brasilense* foliar and seed inoculation improved the morphological indices of maize.

Higher protein content in leaves of treated plants insinuate toward better availability of N-sources as evident by the application of these PGPR (Hassan & Bano, 2015; Nawaz *et al*., 2020). Salt stress induced water deficiency leading to accumulation of carbohydrates, and the observed PGPR induced improvement in soluble sugars was indicative of its role in maintaining osmotic balance (Kumar *et al*., 2020).

Under salt stress, accumulation of reactive oxygen species (ROS) enhanced at greater pace rendering destruction in cells. The enzymetic and non-enzymatic activities of antioxidants play vital role in detoxification mechanism of plants (Hasanuzzaman *et al*., 2020). The observed increases in antioxidant enzymes activities insinuate toward better adaptive strategy which is triggered by positive role of PGPR and (Numan *et al*., 2018; Batool *et al*., 2020). Higher antioxidant activities in treated leaves demonstrate existence of strong antioxidant defence system to cope with physiological disorders related to salt stress (Khan *et al*., 2020).

The PGPR consortium with carrier materials increased phytohormones (IAA, GA_3 and ABA) production in leaves. Phytohormones production by *Bacillus cereus* and *Pseudomonas moraviensis* have been reported in earlier studies (Ozdal *et al*., 2017; Kulkova *et al*., 2023). Results elucidate that changes in phytohormones (IAA and ABA) level reduce the salinity by improving root architecture and biomass of plants (Ahmad *et al*., 2020). Ansari & Ahmad (2019) documented that *Bacillus pumilus* and *Bacillus licheniformis* inoculation enhanced GA production in leaves and exhibited strong growth-promoting activity. Similarly, *Bacillus* spp., rhizobia *Azotobacter* spp. *and Azospirillum* spp. have been reported to improve GA contents (Ei *et al*., 2024). The increment in phytohormones production is associated with better turgidity and stomatal conductance under stress (Seleiman *et al*., 2021; Ahluwalia *et al*., 2021).

Conclusions

Stable carrier based biofertilizer under stressed conditions not only increased colonization of applied PGPR but also recovered soil health by decreasing EC, Na, SAR and Cl and increasing vital nutrients. Suitability of Carriers in formulation is major achievement as it increases survival of bio-inoculants under salt stress and augmented plant to strengthen their osmoprotectants, antioxidative enzymes systems and phytohormones production thereby alleviated osmotic, oxidative and dehydration stresses. The ability and strength of formulation may be increased by using multifunctional PGPR including P-solublisers, N-fixers and phytohormone producers. Similarly, application of this formulation may be tested on different crops growing in saline, saline sodic and sodic soil. Spore producing ability of *Bacillus cereus* enabled it to survive better and this was evidenced by its better performance as bio-inoculants. Further field trials on different crops under salt and dehydration stresses are recommended for future.

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