



Original article

Comparative evaluation of different carrier-based multi-strain bacterial formulations to mitigate the salt stress in wheat

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ABSTRACT

The application of liquid bacterial consortia to soil under natural conditions may fail due to various environmental constraints. In this study, the suitability and efficiency of compost, biogas slurry, crushed corn cob, and zeolite as carriers to support the survival of plant growth-promoting rhizobacteria (PGPR) and improve the performance of multi-strain bacterial consortia to mitigate the effects of salinity stress on wheat under pot conditions were evaluated. The survival of strains of *Pseudomonas putida*, *Serratia ficaria*, and *Pseudomonas fluorescens* labelled with *gusA* was evaluated for up to 90 days. Seeds coated with different carrier-based formulations of multi-strain consortia were sown in pots at three different salinity levels (1.53, 10, and 15 dS m⁻¹). Results showed that salinity stress significantly reduced wheat growth, yield, gas exchange, and ionic and biochemical parameter values, but the 1-aminocyclopropane-1-carboxylate (ACC) deaminase-containing multi-strain consortium used mitigated the inhibitory effects of salinity on plant growth and yield parameters. However, carrier-based inoculation further improved the efficacy of multi-strain consortium inoculation and significantly ($P < 0.05$) increased the growth, yield, and physiological parameters value of wheat at all salinity levels. On the basis of the observed trends in survival and the outcomes of the pot trials, the inoculation of multi-strain consortia in compost and biogas slurry carriers resulted in more successful wheat growth under salinity stress compared to that in the rest of the treatments tested.

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1. Introduction

High salinity is a major environmental stressor affecting agricultural systems and causing food insecurity in most parts of the world (Mustafa et al., 2019). Low-quality irrigation water, arid climates, high temperatures, and the uneven distribution of rainfall are the main causes of soil salinization, especially in semi-arid to

arid regions where the evapotranspiration rate of plants is much higher than the size of the leaching fraction, which results in the accumulation of soluble salts in the plough layer (Rajput et al., 2013). Soil salinization is an environmental issue that is increasing the need for eco-friendly technologies in sustainable agriculture, while the ever-increasing human population is demanding more and securer food (Liu et al., 2019; Majeed and Muhammad, 2019).

In plants, a hyperosmotic potential, nutrient and hormonal imbalances, a reduced photosynthetic rate, relative water content, membrane stability index, stomatal conductance, and intrinsic carbon dioxide concentration in the leaf, and accelerated ethylene production are prominent effects of salt stress, all of which lead to losses in net plant production. Wheat dry biomass also considerably suffers as a result of salt stress because a major portion of the plant's energy is utilized to overcome the harmful effects of high salinity (Costa et al., 2018; Talaat and Shawky, 2014). Therefore, it is imperative to cultivate plants in soils with high salinity

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with the maximum possible output to combat the challenges facing food security.

The use of microbial inoculants containing the plant growth-promoting microorganisms isolated from salt-stressed environments could be an economical solution to increase crop yields by mitigating the salinity stress (Al-Barakah and Sohaib, 2019) in the soil (Ma, 2019; Rajput et al., 2013). According to Malusá et al. (2012), microbial inoculants mainly include plant growth-promoting rhizobacteria (PGPR), nitrogen fixing rhizobia, and arbuscular mycorrhizal fungi (AMF). PGPR support the plants with which they are associated by directly and indirectly combating salt stress. Direct mechanisms by which PGPR enhance crop production include via nitrogen fixation, phosphorus solubilization, siderophore production, phytohormone synthesis, and decreased ethylene production as a result of their 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity, while indirect mechanisms can include antibiotic production, iron chelation, exopolysaccharide (EPS) production, and the synthesis of antifungal metabolites (e.g. chitinase) (Hashem et al., 2019). All of these mechanisms may ultimately result in the improvement of major physiological processes in plants, such as protein synthesis, photosynthesis, respiration, and lipid metabolism (Majeed and Muhammad, 2019; Nadeem et al., 2010a,b; Rana et al., 2012).

Bio-inoculants are formulations that consist of beneficial microbes prepared with suitable and easy to use carrier material. Microbial inoculation is a centuries-old method of crop enhancement, but under field conditions inoculations with a single strain show inconsistent results due to the poor adaptability of single-strain microbial inoculants to varying environmental conditions. Simultaneous plant studies showed that the adaptability of bacterial inoculants could be improved by using mixed inoculants of multiple microbes (co-inoculation and multi-strain inoculation), also termed microbial consortia (Vassilev et al., 2015, Khan et al., 2017).

Multi-strain inoculants can perform better in diverse soils because the microbes included in them usually require a wide range of temperature, pH, and moisture conditions for activities like root colonization, resistance against plant pathogens, metabolic activities, nitrogen fixation, phosphorus solubilisation, and hormone and antibiotic production (Elkoca et al., 2010; Upadhyay et al., 2012). When used in the form of consortia, microbial strains are highly competent and have a broad spectrum of actions without the use of genetic engineering, which makes multi-strain inoculation a more reliable method for the enhancement of crop yield and the healthy growth of plants than other approaches (Ma, 2019; Zahir et al., 2018). However, the selection of suitable combinations of the screened strains is a great challenge in the development of consortia.

The application of a bacterial inoculum (liquid inoculum) to the rhizosphere under natural conditions may fail to enhance crop growth due to various environmental constraints, such as careless handling leading to the dispersal of cells to the atmosphere or groundwater, and the short shelf life of the liquid inoculum. Due to such challenges, the use of bio-fertilizers has been adopted less by farmers than other methods. Therefore, for the successful application of inoculants, particular materials, called carriers, are needed that have the potential to support microbial growth and delivery to the rhizosphere (Zafar-ul-Hye et al., 2019). Carrier-based bacterial inoculants (bio-fertilizers) are highly efficient due to their ease of handling and long-term preservation (El-Fattah et al., 2013).

Carriers may be of organic (e.g., compost, biogas slurry, crushed corn cob, biochar, peat, etc.) or inorganic (e.g., zeolite, perlite, lignite, talc, etc.) origin. The choice of the carrier(s) to use mainly depends on their price and availability. Therefore, in the selection of carrier material the following points need to be considered: the

carrier materials should be easily available, cost-effective, physically and chemically stable, non-toxic to plant growth-promoting microbes, biodegradable and free from pollutants, easy to process, and have a good buffering capacity and high moisture holding capacity (Pacheco-Aguirre et al., 2017). Variations among the microbial strains used is also a major factor in the selection of a carrier. The average cell count in a carrier that has satisfactory results is 10^7 CFU (colony-forming unit) g^{-1} of carrier (Sethi and Adhikary, 2012).

The main aim of studying carrier suitability is to be able to offer a desirable micro-environment to increase the survival and efficiency of the bacteria introduced into the rhizosphere, but no one medium can act as a universal carrier because the performance of a carrier material varies from strain to strain. A good carrier is one that has properties that are suitable for supporting the maximum survival and delivery of microbes from the laboratory to the rhizosphere (Brahmaprakash and Sahu, 2012; Pacheco-Aguirre et al., 2017). Therefore, in this study, the suitability and efficiency of compost, biogas slurry, crushed corn cob, and zeolite as carriers was evaluated to improve the performance of multi-strain bacterial inoculation (by seed-coating) in mitigating the negative impacts of salinity stress on wheat.

2. Materials and methods

In this study, the efficacy of four different carriers (compost, biogas slurry, crushed corn cob, and zeolite) in improving the enhancement of the growth of wheat (variety: 'Faisalabad-2008') by multi-strain bacterial inoculation under saline conditions was evaluated. For this purpose, a survival test under axenic conditions and a pot study were conducted.

2.1. Collection of PGPR strains

Three pre-isolated and characterized ACC deaminase-containing salt-tolerant PGPR strains (W2 = *Pseudomonas putida*; W10 = *Serratia ficaria*; and W17 = *Pseudomonas fluorescens*) were obtained from the Soil Microbiology and Biochemistry Laboratory, Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad, Pakistan, which had already shown their plant growth-promotion ability in terms of their positive behavior to ACC deaminase activity, exopolysaccharide production and phosphate solubilisation, under saline conditions (Nadeem et al., 2010a,b, 2013b).

2.2. Compatibility test

All bacterial strains were investigated for their growth compatibility, as described by Raja et al. (2006). Each isolate was cultured separately in 50 mL of Luria Bertani (LB) medium at 28 ± 1 °C on a shaker at 100 rpm for 48–72 h, and then all strains were cross-streaked on the same LB agar plate. These steps were repeated three times. The cross-streaked plates were incubated at 28 ± 1 °C for 48–72 h, and then examined for the formation of inhibition zones around the colonies.

2.3. Bacterial survival tests in different carriers

To evaluate the survival of the bacterial strains in the compost, biogas slurry, crushed corn cob, and zeolite carriers, strains labeled with glucuronidase (GUS) were used. The selected strains W2 (*Pseudomonas putida*), W10 (*Serratia ficaria*), and W17 (*Pseudomonas fluorescens*) were tagged with the *gusA* gene, following the protocol described by Wilson et al. (1995). The survival of these labeled bacterial strains was monitored at room temperature

(25 ± 2 °C) under axenic conditions. For colony counting, appropriate dilutions of each strain were plated on LB agar plates with the addition of 100 g mL^{-1} spectinomycin, 100 g mL^{-1} XGlcA (5-bromo-4-chloro-3-indolyl- β -D-glucuronide), and 100 g mL^{-1} IPTG (isopropyl- β -D-galactopyranoside). Plates were incubated at 28 ± 1 °C for 3–4 days. The number of blue colonies (CFU g^{-1}) that formed was recorded to determine the average of CFU up to 90 days, which was checked at 30-days intervals (Naveed et al., 2014).

2.4. Inoculum preparation

Each inoculum was prepared in sterilized LB medium and incubated for 3 days at 28 ± 1 °C at 100 rpm in an orbital shaking incubator. Uniform cell density was achieved by using an optical density meter (Den-1 Densitometer, McFarland, UK). Each multi-strain inoculum was prepared using the broth cultures of the W2, W10, and W17 strains in equal proportions, with similar cell populations each (ca. 10^8 cells mL^{-1}).

2.5. Characterization of carriers

The physicochemical properties of the selected carriers (compost, biogas slurry, crushed corn cob, and zeolite) were characterized following standard procedures (Table 1) and data regarding characteristics of compost and biogas slurry was already given in (Irfan et al., 2019). The electrical conductivity (EC) and pH of each of the selected carriers were measured with a compact digital meter (inoLab pH/Cond Level 1). Equal amounts of each solid carrier and distilled water were mixed and stirred thoroughly to form a paste, and the pH of the paste was then determined (Page, 1982). The water-holding capacity of a carrier was determined on a dry-mass basis (Somasegaran and Hoben, 1994). The C:N ratio was determined by the standard method described by Jimenez and Ladha (1993). The total phosphorous and potassium content of all carrier materials was also analyzed using the triple-acid digestion method (Page, 1982).

2.6. Pot trial

Carrier materials were processed (dried, ground, sieved, and sterilized by autoclaving) and inoculated (hereinafter: InCCC = inoculated crushed corn cob; InCOM = inoculated compost; InBGS = inoculated biogas slurry; and InZEO = inoculated zeolite) with the multi-strain inoculum (100 mL kg^{-1}), and were then incubated overnight. Each carrier material was also treated with sterilized broth to create un-inoculated control carriers (hereinafter: CCC = un-inoculated crushed corn cob; COM = un-inoculated compost; BGS = un-inoculated biogas slurry; and ZEO = un-inoculated zeolite) to segregate the effects of the carrier from those of inoculation. Before seed-coating, wheat seeds were surface-sterilized by

dipping them in 70% ethanol for 1 min and 3.5% sodium hypochlorite for 5 min, followed by washing them 4 times with autoclaved distilled water. For seed-coating, seed dressing was carried out with the different inoculated carriers mixed with clay and 10% sugar (sucrose) solution. For un-inoculated controls (Ctrl), seeds were coated following the same procedures, but using the sterilized inoculum in each of the different carriers. Another treatment (InLIQ: Liquid inoculum as inoculated control) with multi-strain inoculation but no carrier material was also tested, wherein wheat seeds were dipped in the liquid inoculum for 15 min. Coated seeds were sown in pots of soil at different salinity levels. Three levels of salinity, 1.53 (original), 10, and 15 dS m^{-1} , were used for this pot trial. Calculated amounts of NaCl salt were used to develop each of the three chosen salinity levels in the pots. All treatments were repeated three times.

2.7. Soil analysis

The physicochemical properties of the soil were estimated by the following standard procedures (Table 2). Soil texture was estimated through Bouyoucos' hydrometer method (Gee and Bauder, 1986). The soil's textural class was designated using the International Textural Triangle. A saturated paste was prepared and the soil saturation percentage was calculated following the procedure of Rhoades et al. (1989).

The pH of the saturated soil paste and electrical conductivity (EC) were measured with a digital compact meter (inoLab pH/Cond Level 1). To estimate cation exchange capacity (CEC), followed the methodology of the U.S. Salinity Laboratory Staff (Richards, 1969).

Soil organic matter contents were determined according to the method described by Moodie et al. (1951). Nitrogen contents were determined by Ginning and Hibbard's sulfuric acid digestion method, and distillation was done with a macro Kjeldahl's apparatus (Jackson, 1958). The available phosphorus in the soil was estimated using a spectrophotometer (Milton Roy Company) at a wavelength of 880 nm and a standard curve (Watanabe and Olsen, 1965).

2.8. Plant analysis

At the booting stage, gas exchange parameters, including the photosynthetic rate (A), transpiration rate (E), intrinsic CO_2 concentration (C_i), stomatal conductance (g_s), photosynthetic water use efficiency (A/E), and intrinsic water use efficiency (A/g_s), were measured using a CIRAS-3 portable photosynthesis system (PP System, Amesbury, MA, USA) with a PLC3 universal leaf cuvette, taking measurements on both sides of the flag leaves. The cuvette was provided with light from a light-emitting diode (LED) with a photon flux of $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$, at ambient leaf temperature and $390 \mu\text{mol mol}^{-1} \text{CO}_2$. After gas exchange parameter data had been collected, the flag leaves were collected and further analyzed to

Table 1
Physicochemical properties of carrier materials (Irfan et al., 2019), (nd: not detected).

Characteristics	Unit	Carrier materials			
		Crushed corn cob	Compost	Biogas slurry	Zeolite
pH _{1:1}	–	6.52	6.79	7.84	7.42
EC _{1:2}	dS m^{-1}	0.93	2.09	7.13	0.24
WHC	%	82.63	60.01	52.60	36.29
Inherent moisture capacity	%	8.31	9.72	8.86	2.21
Total C	%	44.01	14.93	50.12	nd
Total N	%	0.40	0.94	5.81	nd
Total P	%	32.90	1.20	1.05	nd
Total K	%	0.023	1.40	1.44	1.50
C:N	–	110.03	15.88	8.63	nd

Table 2
Physicochemical properties of pot soil.

Characteristics	Unit	Pot soil
Textural class	–	Sandy clay loam
Saturation percentage	%	33.30
pH _s	–	7.84
EC _e	dS m ⁻¹	1.53
CEC	cmol _c kg ⁻¹	7.18
Organic matter	%	0.68
Total N	%	0.046
Available P	mg kg ⁻¹	9.81
Extractable K	mg kg ⁻¹	178

determine their relative water content, membrane stability index, and chlorophyll *a*, chlorophyll *b*, carotenoid, and proline content. The relative water content (RWC) in the leaves and their membrane stability index (MSI) were estimated as described by [Mayak et al. \(2004\)](#) and [Sairam \(1994\)](#), respectively. Chlorophyll *a*, chlorophyll *b*, and carotenoids were estimated by spectrophotometer method ([Arnon, 1949](#)). The proline content were determined according to the method described by [Bates et al. \(1973\)](#).

Sodium (Na⁺) and potassium (K⁺) concentrations were analyzed with a flame photometer, as described by [Richards \(1969\)](#). The color (SPAD value) of the flag leaves was observed with a portable chlorophyll meter (SPAD-501) before grain formation.

The total content of nitrogen, phosphorus, and potassium was determined after digestion. The plant samples were digested according to the method of [Wolf \(1982\)](#). The nitrogen contents were determined by Kjeldahl's method. The phosphorus contents were determined by spectrophotometry using a standard curve and Barton's reagent. The potassium content was measured using a flame photometer. A series of standards was prepared and the standard curve was drawn for each element. The crude protein (CP) content was estimated by multiplying the grain nitrogen content by a factor of 6.25 ([Thimmaiah, 2004](#)).

At maturity, the plant growth parameters were assessed following standard methods. Plant growth performance was assessed by determining the plant height (cm), spike length (cm), root length (cm), root weight (g), total biomass (g), straw yield (g), 100-grain weight (g), number of spikelets per spike, and number of tillers per plant.

2.9. Statistical analyses

Data from the survival test were processed and presented through SigmaPlot 10 ([Systat Software Inc., 2006](#)). Pot trial data were subjected to analysis of variance (ANOVA) ([Steel et al., 1997](#)) with a fully factorial experimental design. All pairwise comparisons between treatment means were made using Fisher's LSD test, at a significance level of $P < 0.05$ ([Williams and Abdi, 2010](#)).

3. Results

3.1. Survival of bacterial strains in different carriers

The results of the survival test ([Fig. 1](#)) showed that strain W17 reached the maximum counts observed (18.28×10^8 CFU g⁻¹) in compost, followed by W17 in biogas slurry (14.33×10^8 CFU g⁻¹), and W10 in compost (13.33×10^8 CFU g⁻¹) after 90 days. Generally, the bacterial counts decreased with time in all carriers throughout the study period, although strain W2 maintained about constant and maximal counts after up to 60 days in compost. Crushed corn cob resulted in minimum counts for all studied strains. Among all the studied carriers, compost and biogas slurry were the best, and supported the maximum counts of viable bacteria for up to 90 days.

3.2. Compatibility test

When the inhibition zones on the inoculated plates were observed, all of the studied strains were found to be compatible with each other, as described by [Raja et al. \(2006\)](#).

3.3. Physiological traits and photosynthetic activity of wheat

Results ([Table 3](#)) showed that, under salinity stress (10 and 15 dS m⁻¹), there were significant decreases in the CO₂ assimilation or photosynthetic rate (up to 47.85 and 70.96%), transpiration rate (up to 33.63 and 48.68%), stomatal conductance (up to 23.39 and 38.98%), and water use efficiency (up to 21.36 and 43.27%) of the wheat compared to those in the non-saline condition (1.53 dS m⁻¹), respectively. However, carrier-based inoculation further improved the ability of the tested consortium to alleviate salinity stress, and significantly increased the photosynthetic rate, transpiration rate, stomatal conductance, and water use efficiency of wheat at all salinity levels compared to those in each of the respective un-inoculated controls and with the liquid inoculation.

In the non-saline condition (1.53 dS m⁻¹), the compost-based inoculation increased the photosynthetic rate (significantly, up to 57.76 and 23.51%), transpiration rate (significantly, up to 35.67 and 13.53%), and stomatal conductance (up to 29.04 and 11.31%) compared to those in the un-inoculated control and with the liquid inoculum, respectively, while zeolite-based inoculation increased the water use efficiency up to 17.98 and 8.90% compared to that in the un-inoculated control and with the liquid inoculum, respectively. At the salinity of 10 dS m⁻¹, compost-based inoculation increased the photosynthetic rate (up to 92.41 and 42.06%), transpiration rate (up to 64.68 and 27.85%), and stomatal conductance (significantly, up to 42.92 and 18.75%) compared to those in the un-inoculated control and with the liquid inoculum, respectively, while zeolite-based inoculation increased the water use efficiency up to 17.69 and 8% compared to the un-inoculated control and with the liquid inoculum, respectively. At the salinity of 15 dS m⁻¹, compost-based inoculation significantly increased the photosynthetic rate (up to 145.45 and 61.18%), transpiration rate (up to 97.27 and 40.21%), and stomatal conductance (up to 41.67 and 18.42%) compared to those in the un-inoculated control and with the liquid inoculum, respectively, while, zeolite-based inoculation also increased the water use efficiency up to 25.56 and 9.01% compared to that in the un-inoculated control and with the liquid inoculum, respectively.

A reduced intrinsic CO₂ concentration (*C_i*) and plant water use efficiency (*A/E*) are associated with reduced plant stomatal conductance (*g_s*). Under salinity stress (10 and 15 dS m⁻¹), the intrinsic CO₂ concentration (*C_i*) significantly increased compared to that in the non-saline condition (1.53 dS m⁻¹) by up to 45.45 and 67.27%, respectively. In the non-saline condition (1.53 dS m⁻¹), biogas slurry-based inoculation decreased the intrinsic CO₂ concentration by up to 9.55 and 5.24% compared to that in the un-inoculated control and with the liquid inoculum, respectively. At the salinity of 10 dS m⁻¹, compost-based inoculation significantly decreased the intrinsic CO₂ concentration by up to 54.69 and 41.06% compared to that in the un-inoculated control and with the liquid inoculum, respectively. At the salinity of 15 dS m⁻¹, compost-based inoculation also significantly decreased the intrinsic CO₂ concentration by up to 46.2 and 31.25% compared to that in the un-inoculated control and with the liquid inoculum, respectively.

Under salinity stress (10 and 15 dS m⁻¹), the membrane stability index (MSI) (by up to 21.6 and 33.48%) and relative water content (RWC) (by up to 29.2 and 45.15%) were significantly decreased compared to those in the non-saline condition (1.53 dS m⁻¹), respectively. In the non-saline condition (1.53 dS m⁻¹), compost-

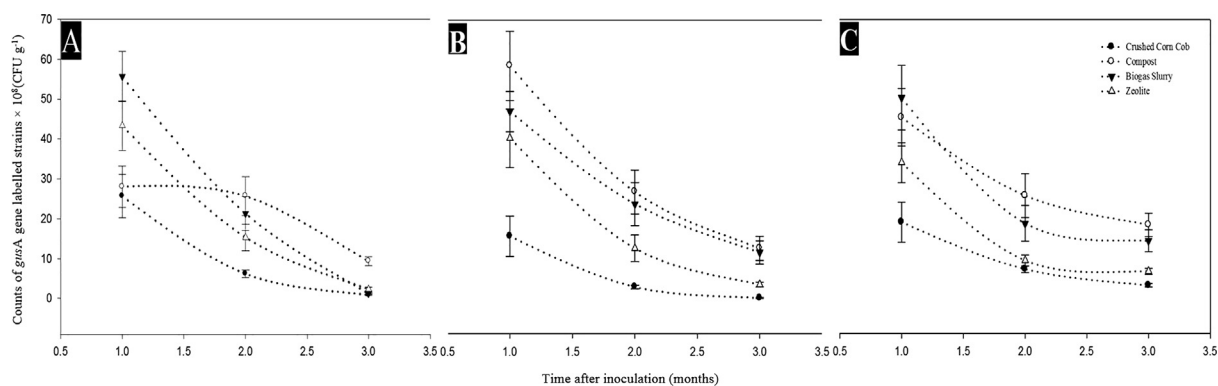


Fig. 1. Survival trend (in terms of culturable colony counts) of *gusA* gene labelled *Pseudomonas putida* (A), *Serratia ficaria* (B) and *Pseudomonas fluorescens* (C) in different carrier materials up to 90 days at 25 ± 2 °C.

Table 3

Effect of different carrier based inoculations of bacterial consortia on physiological traits of wheat at different salinity levels in a pot trial. Means sharing similar letter(s) are statistically non-significant at $P < 0.05$ according to LSD test.

Treatments	NaCl Salinity (dS m^{-1})																
	1.53			10			15			1.53			10			15	
	A ($\mu\text{mole CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)			E ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$)			Ci ($\mu\text{mol mol}^{-1}$)			gs ($\mu\text{mole H}_2\text{O m}^{-2} \text{ s}^{-1}$)							
Ctrl	10.10 c	5.27 i	2.93 j	2.62 d-f	1.74 h-k	1.34 k	220.0 gh	320.0b-d	368.0 a	295.0 d-f	226.0 ij	180.0 k					
InLIQ	12.90 b	7.13 d-f	4.47 ij	3.13 bc	2.24 fg	1.89 gh	210.0 gh	246.0 fg	288.0 de	342.0 a-c	272.0 e-h	215.3 i-k					
InCCC	13.20 b	7.70 d	4.80 i	3.13 bc	2.39 ef	1.94 gh	208.0 gh	235.0f-h	276.0 ef	347.3 ab	278.0 e-g	219.0 i-k					
CCC	10.13 c	5.30 hi	2.93 j	2.63 d-f	1.75 h-j	1.36 jk	219.0 gh	317.7 cd	366.0 a	296.7 d-f	227.3 ij	182.0 k					
InCOM	15.93 a	10.13 c	7.20 de	3.55 a	2.86 cd	2.65 de	200.0 h	145.0 j	198.0 hi	380.7 a	323.0 bd	255.0 f-i					
COM	10.77 c	5.57 e-i	3.07 j	2.75 c-e	1.78 hi	1.40 i-k	216.0 gh	311.0 de	359.0 a-c	303.7b-e	233.0 hi	185.0 jk					
InBGS	15.53 a	9.97 c	7.03 d-g	3.46 ab	2.82 cd	2.59 d-f	199.0 h	154.0 j	202.0 h	372.0 a	313.3 b-e	251.0 g-i					
BGS	10.63 c	5.53 f-i	3.07 j	2.76 c-e	1.83 h	1.40 i-k	218.0 gh	315.0 de	361.0 ab	301.0c-e	234.3 g-i	186.0 jk					
InZEO	15.30 a	9.80 c	6.93 d-h	3.41 ab	2.78 c-e	2.55 d-f	201.0 h	157.0 ij	210.0 gh	370.0 a	308.7b-e	245.0 g-i					
ZEO	10.50 c	5.43 g-i	3.03 j	2.69 de	1.79 hi	1.39 j	217.0 gh	314.0 de	360.0 ab	300.7 c-e	232.3 hi	184.0 jk					
LSD value	1.6566			0.3958			41.375			43.925							
	A/E ($\mu\text{mole CO}_2 \text{ mmol}^{-1} \text{ H}_2\text{O}$)			A/gs ($\mu\text{mole CO}_2 \text{ mmol}^{-1} \text{ H}_2\text{O}$)			MSI (%)			RWC (%)							
Ctrl	3.860 a-d	3.035 d-g	2.190 g	0.035 a-c	0.024 e-i	0.016 i	45.2 ef	35.5 j	30.1 k	74.9 b	53.0 g-i	41.1 j					
InLIQ	4.182 a-c	3.308 c-f	2.522 fg	0.038 ab	0.026 d-g	0.020 g-i	53.7 b	40.1 hi	35.7 j	79.9 ab	59.4 c-g	49.2 l					
InCCC	4.204 a-c	3.284 c-f	2.574 e-g	0.038 ab	0.028 c-g	0.022 f-i	54.7 b	40.9 gh	36.8 j	81.1 ab	60.1 c-f	49.8 hi					
CCC	3.880 a-d	3.036 d-g	2.209 g	0.034 a-c	0.024 e-i	0.016 i	45.8 de	36.4 j	31.1 k	75.2 b	53.0 g-i	41.2 j					
InCOM	4.490 ab	3.548 b-e	2.724 e-g	0.042 a	0.031 b-e	0.028 f	61.6 a	49.5 c	42.9 fg	85.4 a	65.2 c	58.2 d-g					
COM	3.920 a-d	3.120 d-g	2.201 g	0.036 a-c	0.024 d-h	0.017 hi	49.2 c	37.8 ij	32.3 k	76.5 b	54.3 f-i	42.2 j					
InBGS	4.502 ab	3.552 b-e	2.733 e-g	0.042 a	0.032 b-d	0.028 c-g	59.7 a	48.3 cd	42.1 gh	85.1 a	64.6 cd	57.9 e-g					
BGS	3.924 a-d	3.072 d-g	2.217 g	0.036 a-c	0.024 e-i	0.017 h-i	49.2 c	38.0 ij	30.4 k	75.8 b	54.3 f-i	42.0 j					
InZEO	4.554 a	3.572 a-e	2.750 e-g	0.042 a	0.032 b-d	0.028 c-f	59.4 a	46.5 de	39.6 hi	84.1 a	63.4 c-e	56.5 f-h					
ZEO	3.915 a-d	3.055 d-g	2.201 g	0.035 a-c	0.024 e-i	0.017 hi	47.1 c-e	36.3 j	32.3 k	76.5b	53.9 f-i	41.6 j					
LSD value	1.0001			0.0078			2.6243			6.5286							

based inoculation significantly increased the membrane stability index (up to 36.21 and 14.78%) and relative water content (up to 14.07 and 6.91%) compared to those in the un-inoculated control and with the liquid inoculum, respectively. At the salinity of 10 dS m^{-1} , compost-based inoculation increased the membrane stability index (up to 39.62 and 23.35%) and relative water content (up to 22.95 and 9.81%) compared to those in the un-inoculated control and with the liquid inoculum, respectively. At the salinity of 15 dS m^{-1} , compost-based inoculation also increased the membrane stability index (up to 42.57 and 20.11%) and relative water content (significantly, up to 41.66 and 18.25%) compared to those in the un-inoculated control and with the liquid inoculum, respectively.

3.4. Biochemical traits of wheat

Results (Table 4) revealed that, under salinity stress (10 and 15 dS m^{-1}), there were significant decreases in the chlorophyll *a* (up to 43.85 and 74.66%), chlorophyll *b* (up to 32.67 and 66.51%),

carotenoid (up to 33.6 and 47.38%), total chlorophyll (up to 39.7 and 70%), and crude protein content (up to 31.13 and 49.55%), as well as in the ratio of chlorophyll *a* to chlorophyll *b* (up to 16.64 and 23.72%) and the SPAD value (up to 22 and 28%), compared to those in the non-saline condition (1.53 dS m^{-1}), respectively.

In the non-saline condition (1.53 dS m^{-1}), compost-based inoculation increased the chlorophyll *b* (up to 7.04 and 3.22%), carotenoid (up to 26.29 and 5.68%), total chlorophyll (up to 6.65 and 2.76%), and crude protein content (significantly, up to 3.72 and 2.27%), and the SPAD value (up to 20 and 7.14%), compared to those in the un-inoculated control and with the liquid inoculum, respectively, while biogas slurry-based inoculation increased the chlorophyll *a* content by up to 4.37 and 2.22% compared to that in the un-inoculated control and with the liquid inoculum, respectively.

At the salinity of 10 dS m^{-1} , biogas slurry-based inoculation increased the chlorophyll *b* content (significantly, up to 39.82 and 14.77%), ratio of chlorophyll *a* to chlorophyll *b* (up to 29.08 and 9.56%), and SPAD value (up to 34.19 and 9.02%) compared to

Table 4
Effect of different carrier based inoculations of bacterial consortia on biochemical traits of wheat at different salinity levels in a pot trial. Means sharing similar letter(s) are statistically non-significant at $P < 0.05$ according to LSD test.

Treatments	NaCl Salinity (dS m ⁻¹)																	
	1.53			10			15			1.53			10			15		
	Chlorophyll 'a' (mg g ⁻¹)			Chlorophyll 'b' (mg g ⁻¹)			Chlorophyll 'a'/Chlorophyll 'b'			Carotenoids (mg g ⁻¹)								
Ctrl	2.739 a	1.538 ef	0.694 h	1.359 ab	0.915 d	0.455 f	2.016 bc	1.681 c-g	1.538 g	0.300 b	0.199 d	0.158 e						
InLIQ	2.797 a	2.163 bc	1.291 g	1.409 ab	1.115 c	0.580 ef	1.986b-e	1.980 b-e	2.252 b	0.359 a	0.246 c	0.213 d						
InCCC	2.797 a	2.212b	1.317 fg	1.412 ab	1.116 c	0.586 ef	1.980b-e	1.987 b-e	2.242 b	0.359 a	0.250 c	0.216 d						
CCC	2.740 a	1.540 ef	0.717 h	1.379 ab	0.950 d	0.460 f	2.008 bc	1.639 c-g	1.577 fg	0.294b	0.197 d	0.134 e						
InCOM	2.857 a	2.765 a	1.949 cd	1.455 a	1.272 b	0.703 e	1.979b-e	2.159 b	2.781 a	0.379 a	0.315 b	0.316 b						
COM	2.764 a	1.558 e	0.743 h	1.384 ab	0.927 d	0.468 f	2.010 bc	1.687 c-g	1.584 fg	0.296b	0.196 d	0.153 e						
InBGS	2.859 a	2.751 a	1.949 cd	1.453 a	1.280 b	0.699 e	1.969b-f	2.169 b	2.829 a	0.368 a	0.303 b	0.301 b						
BGS	2.758 a	1.556 e	0.742 h	1.382 ab	0.928 d	0.465 f	2.000b-d	1.688 c-g	1.599 e-g	0.296b	0.196 d	0.152 e						
InZEO	2.842 a	2.748 a	1.928 d	1.444 a	1.277 b	0.695 e	1.983b-e	2.158 b	2.788 a	0.366 a	0.303 b	0.298 b						
ZEO	2.752 a	1.552 e	0.725 h	1.371 ab	0.928 d	0.474 f	2.015 bc	1.681 c-g	1.607 d-g	0.295b	0.196 d	0.151 e						
LSD value	0.2256			0.1515			0.3934			0.0285								
	Total chlorophyll (mg g ⁻¹)			SPAD value			Protein ((μmole g ⁻¹)			Crude protein (%)								
Ctrl	4.399 a-c	2.653 f	1.319 h	50.0 a-f	39.0b-g	36.0 g	0.741f	1.188 a	1.192 a	13.46 c	9.27 g	6.88 j						
InLIQ	4.565 a-c	3.524 d	2.084 g	56.0 ab	48.0 a-g	38.7c-g	0.699 fg	1.075 bc	1.087 b	13.65 bc	10.02 e	7.44 i						
InCCC	4.568 a-c	3.578 d	2.118 g	56.3 a	48.7 a-g	39.3b-g	0.699 fg	1.085 b	1.085 b	13.71 a-c	10.06 e	7.25 i						
CCC	4.414 a-c	2.687 ef	1.310 h	50.7 a-e	39.3b-g	36.3 g	0.741 f	1.194 a	1.216 a	13.48 c	9.33 fg	7.06 j						
InCOM	4.691 a	4.351 bc	2.968 e	60.0 a	51.0 a-d	42.0 b-g	0.640 h	0.978 de	1.012 de	13.96 a	10.79 d	8.13 h						
COM	4.444 a-c	2.682 ef	1.364 h	51.7 a-c	40.3b-g	38.0 d-g	0.747 f	1.181 a	1.159 a	13.52 c	9.56 f	7.00 j						
InBGS	4.680 a	4.333 c	2.949 ef	59.0 a	52.3 ab	40.7 b-g	0.645 gh	0.962 e	1.006 de	13.98 a	10.71 d	7.75 h						
BGS	4.436 a-c	2.680 ef	1.359 h	51.3 a-d	40.0b-g	37.3 e-g	0.736 f	1.177 ab	1.164 a	13.54 c	9.54 fg	7.19 j						
InZEO	4.652 ab	4.327c	2.920 ef	58.3 a	50.3 a-e	39.7 b-g	0.652 gh	0.999 de	1.021 cd	13.90 ab	10.67 d	7.81 h						
ZEO	4.418 a-c	2.676 ef	1.350 h	51.0 a-d	39.7b-g	36.7 fg	0.738 f	1.200 a	1.186 a	13.50 c	9.40 fg	6.94 j						
LSD value	0.3095			13.454			0.0582			0.2746								

those in the un-inoculated control and with the liquid inoculum, respectively, while compost-based inoculation significantly increased the chlorophyll *a* (up to 79.75 and 27.83%), total chlorophyll (up to 64.04 and 23.47%), carotenoid (up to 57.83 and 27.76%), and crude protein content (up to 16.4 and 7.68%) compared to those in the un-inoculated control and with the liquid inoculum, respectively.

At the salinity of 15 dS m⁻¹, compost-based inoculation also increased the chlorophyll *a* (significantly, up to 180.81 and 50.96%), chlorophyll *b* (up to 54.50 and 21.24%), carotenoid (significantly, up to 99.77 and 48.52%), total chlorophyll (significantly, up to 124.97 and 42.43%), and crude protein content (significantly, up to 16.56 and 7.03%), as well as the SPAD value (up to 16.67 and 8.61%), compared to those in the un-inoculated control and with the liquid inoculum, respectively, while biogas slurry-based inoculation significantly increased the ratio of chlorophyll *a* to chlorophyll *b* by up to 83.98 and 25.62% compared to that in the un-inoculated control and with the liquid inoculum, respectively.

Under salinity stress (10 and 15 dS m⁻¹), the proline content in the leaves of wheat was significantly increased compared to that in the non-saline condition (1.53 dS m⁻¹) by up to 44.70 and 60.86%, respectively. However, the bacterial consortium decreased the proline content by up to 5.66, 9.51, and 8.85% compared to those in the un-inoculated controls at salinities of 1.53, 10, and 15 dS m⁻¹, respectively. At the original salinity (1.53 dS m⁻¹), liquid inoculation did not significantly change the proline content from that in the un-inoculated control. However, carrier-based inoculation further improved the efficacy of the consortium, and significantly decreased the proline content at all salinity levels compared to that in the un-inoculated control and with the liquid inoculation. In the non-saline condition (1.53 dS m⁻¹), compost-based inoculation decreased the proline content by up to 13.71 and 8.44% compared to that in the un-inoculated control and with the liquid inoculum, respectively. At the salinity of 10 dS m⁻¹, biogas slurry-based inoculation significantly reduced the proline content in the leaves by up to 19.02 and 7.07% compared to that in the un-inoculated control and with the liquid inoculum, respectively. At the salinity of

15 dS m⁻¹, biogas slurry-based inoculation also significantly decreased the proline content by up to 15.66 and 7.45% compared to that in the un-inoculated control and with the liquid inoculum, respectively.

3.5. Chemical traits of wheat

The results obtained for the chemical traits (Table 5) of wheat showed that, under salinity stress (10 and 15 dS m⁻¹), there were decreases in the K⁺/Na⁺ ratio in the leaves (significantly, by up to 63.85 and 78.72%), nitrogen content in straw (significantly, up to 8 and 23.2%), phosphorous content in straw (up to 16 and 32%), nitrogen content in grain (significantly, up to 31.16 and 49.30%), phosphorous contents in grain (up to 26 and 52%), and potassium content in grain (up to 25.45 and 24.24%) compared to those in the non-saline condition (1.53 dS m⁻¹), respectively.

In the non-saline condition (1.53 dS m⁻¹), compost-based inoculation increased the K⁺/Na⁺ ratio in the leaves (up to 15.53 and 9.17%), nitrogen content in straw (up to 32.35 and 13.01%), phosphorous content in straw (up to 28.38 and 10.34%), nitrogen content in grain (significantly, up to 3.72 and 2.29%), phosphorous content in grain (significantly, up to 37.09 and 11.29%), and potassium content in grain (up to 4.66 and 1.78%) compared to those in the un-inoculated control and with the liquid inoculum, respectively.

At the salinity of 10 dS m⁻¹, compost-based inoculation increased the K⁺/Na⁺ ratio in the leaves (significantly, up to 78.52 and 29.73%), nitrogen content in straw (significantly, up to 30.43 and 11.94%), phosphorous content in straw (up to 23.44 and 8.33%), nitrogen content in grain (significantly, up to 16.4 and 8.13%), phosphorous content in grain (up to 34.55 and 8.89%), and potassium content in grain (significantly, up to 19.84 and 8.89%) compared to those in the un-inoculated control and with the liquid inoculum, respectively.

At the salinity of 15 dS m⁻¹, compost-based inoculation also increased the K⁺/Na⁺ ratio in the leaves (significantly, up to 111.65 and 11.49%), nitrogen content in straw (significantly, up

Table 5

Effect of different carrier based inoculations of bacterial consortia on chemical traits of wheat at different salinity levels in a pot trial. Means sharing similar letter(s) are statistically non-significant at $P < 0.05$ according to LSD test.

Treatments	NaCl Salinity (dS m ⁻¹)								
	1.53			10			15		
	K ⁺ /Na ⁺ in leaves			Nitrogen contents in straw (%)			Phosphorous contents in straw (%)		
Ctrl	2.96 a-c	1.07 gh	0.63 k	1.25 fg	1.15 h	0.96 i	0.25 d-h	0.21 h-l	0.17 m
InLIQ	3.15 a	1.47 ef	0.95 h-j	1.46 bc	1.34 e	1.18 h	0.29 a-c	0.24 d-j	0.20 j-m
InCCC	3.22 a	1.53 de	0.99 a-d	1.51 b	1.37 de	1.20 gh	0.29 ab	0.24 d-i	0.21 i-m
CCC	2.99 a-c	1.10 gh	0.66 jk	1.26 f	1.16 h	0.97 i	0.25 c-h	0.22 g-k	0.17 m
InCOM	3.42 a	1.92 c	1.34 e-g	1.65 b	1.50 b	1.41 cd	0.32 a	0.26 b-d	0.23 d-j
COM	3.02 a-c	1.16 f-h	0.67 b-e	1.28 f	1.18 h	0.99 i	0.26 a-d	0.22 e-j	0.18 k-m
InBGS	3.39 a	1.85 cd	1.23 e-h	1.62 b	1.48 b	1.38 de	0.31 a	0.26 a-d	0.22 e-j
BGS	3.03 ab	1.16 f-h	0.69 i-k	1.27 f	1.18 h	0.99 i	0.26 b-f	0.22 f-j	0.18 k-m
InZEO	3.35 a	1.80 cd	1.23 e-h	1.62 b	1.49 b	1.37 de	0.31 a	0.26 b-f	0.22 f-j
ZEO	2.99 a-c	1.11 gh	0.66 jk	1.26 f	1.17 h	0.97 i	0.25 b-g	0.22 g-k	0.18 lm
LSD value	0.3191			0.1038			0.0381		
Treatments	1.53			10			15		
	Nitrogen contents in grain (%)			Phosphorous contents in grain (%)			Potassium contents in grain (%)		
	Ctrl	2.15 c	1.48 g	1.09 j	0.50 c-e	0.37 h	0.24 i	1.65 c	1.23 j
InLIQ	2.18 bc	1.60 e	1.18 i	0.62 b	0.45 e-g	0.35 h	1.69 a-c	1.35 g	1.33 gh
InCCC	2.19 a-c	1.61 e	1.19 i	0.63 b	0.45 d-f	0.36 h	1.70 ab	1.37 fg	1.34 g
CCC	2.16 c	1.49 fg	1.10 j	0.51 cd	0.37 h	0.24 i	1.65 c	1.25 ij	1.26 ij
InCOM	2.23 a	1.73 d	1.27 h	0.69 a	0.49 c-e	0.40 f-h	1.72 a	1.47 ef	1.41 b-d
COM	2.16 c	1.53 f	1.13 j	0.53 c	0.38 h	0.25 i	1.65 bc	1.28 i	1.29 hi
InBGS	2.24 a	1.71 d	1.25 h	0.68 ab	0.49 c-e	0.39 gh	1.72 a	1.44 de	1.36 fg
BGS	2.17 c	1.53 fg	1.12 j	0.52 c	0.38 h	0.25 i	1.66 bc	1.27 ij	1.28 i
InZEO	2.22 ab	1.71 d	1.24 h	0.68 ab	0.48 c-e	0.39 gh	1.72 a	1.45 fg	1.38 b-e
ZEO	2.16 c	1.50 fg	1.11 j	0.51 c	0.37 h	0.24 i	1.65 c	1.26 ij	1.26 ij
LSD value	0.0440			0.0599			0.0485		

to 47.22 and 19.49%), phosphorous content in straw (up to 33.33 and 15%), nitrogen content in grain (significantly, up to 16.56 and 7.63%), phosphorous content in grain (up to 67.61 and 14.28%), and potassium content in grain (significantly, up to 12.80 and 6.02%) compared to those in the un-inoculated control and with the liquid inoculum, respectively.

3.6. Physical traits of wheat

Results (Table 6) revealed that, under salinity stress (10 and 15 dS m⁻¹), there were decreases in the plant height (by up to 26.74 and 31.40%), spike length (up to 15.04 and 20.98%), root length (up to 24.79 and 49.77%), root weight (significantly, up to

Table 6

Effect of different carrier based inoculations of bacterial consortia on physical traits of wheat at different salinity levels in a pot trial. Means sharing similar letter(s) are statistically non-significant at $P < 0.05$ according to LSD test.

Treatments	NaCl Salinity (dS m ⁻¹)																	
	1.53			10			15			1.53			10			15		
	Plant height (cm)			Spike length (cm)			Root length (cm)			No of tillers/plant								
Ctrl	86.0 a-c	63.0 g-i	59.0 i	11.8 a	10.0 cd	9.3 d	21.9 a-c	16.5 fg	11.0 h	4.3 a-c	3.3c-e	2.3 e						
InLIQ	88.8 a	76.5 d-f	69.3 e-g	11.8 a	11.1 a-c	10.4 cd	23.7 ab	18.6 c-f	13.8 gh	5.0 a-c	3.7 b-e	3.3 c-e						
InCCC	89.2 a	77.2 d-f	70.3 e-g	11.8 a	11.1 a-c	10.5 b-d	24.1 ab	18.9 cf	14.1 gh	5.3 a-c	4.0 a-e	3.7 b-e						
CCC	86.2 a-c	63.7 g-i	60.0 i	11.8 a	10.1 cd	9.3 d	21.8 a-c	16.8 e-g	11.3 h	4.3 a-e	3.3 c-e	2.3 e						
InCOM	93.0 a	87.0 ab	79.3 b-d	11.8 a	11.9 a	11.0 a-c	24.8 a	21.2 a-c	16.7 f-g	6.0 a	4.7 a-d	4.0 a-e						
COM	87.3 ab	64.7 g-i	61.0 hi	11.8 a	10.3 cd	9.6 d	22.0 a-c	16.9 d-g	11.4 h	4.7 a-d	3.7 b-e	2.7 de						
InBGS	92.0 a	87.3 ab	78.3 c-e	11.8 a	11.9 a	11.0 a-c	24.4 ab	20.9 b-d	15.9 fg	5.7 ab	4.7 a-d	4.0 a-e						
BGS	87.2 ab	64.0 g-i	60.7 i	11.8 a	10.2 c-d	9.5 d	22.0 a-c	17.1 d-g	11.8 h	4.7 a-d	3.3 c-e	2.7 de						
InZEO	91.3 a	86.3 a-c	77.0 d-f	11.8 a	11.7 ab	10.9 a-c	24.5 ab	20.6 b-e	16.4 fg	5.3 a-c	4.3 a-e	3.7 b-e						
ZEO	86.3 a-c	64.0 g-i	60.7 i	11.8 a	10.1 cd	9.4 d	22.0 a-c	16.8 e-g	11.1 h	4.3 a-e	3.3 c-e	2.3 e						
LSD value	8.5519			1.2335			3.9526			2.0002								
Treatments	1.53			10			15			1.53			10			15		
	No. of spikelets/spike			Total biomass (g)			Root weight (g)			100 grain's weight (g)								
	Ctrl	19.7 a-e	15.0 f-j	12.0 j	31.0 b	18.3 fg	16.6 g	7.5 a	5.7 b-f	4.2 g	4.1 d	3.5 j-l	3.2 n					
InLIQ	21.3 ab	17.3 c-f	14.0 f-j	39.9 a	24.1 c-e	22.4 ef	7.7 a	6.9 a-d	5.4 d-g	4.3 bc	3.8 gh	3.7 hi						
InCCC	21.7 a	17.7 b-f	15.0 f-j	40.1 a	24.8 c-e	22.7 d-f	7.8 a	6.9 a-c	5.5 c-g	4.3 b	3.9 ef	3.8 fg						
CCC	19.7 a-e	15.3 f-j	12.3 ij	31.2 b	19.0 fg	16.9 g	7.5 a	5.7 b-f	4.2 g	4.2 d	3.6 jk	3.3 m						
InCOM	22.7 a	20.3 a-c	17.0 c-f	43.3 a	30.9 b	28.3 bc	8.0 a	7.8 a	7.3 a	4.6 a	4.2 b-d	4.0 e						
COM	20.3 a-c	16.0 e-i	13.0 g-j	32.0 b	19.2 fg	17.4 g	7.5 a	5.8 b-e	4.2 fg	4.2 cd	3.6 ij	3.4 k-m						
InBGS	22.3 a	20.0 a-d	16.7 c-g	42.5 a	30.5 b	27.4 b-d	8.0 a	7.7 a	6.9 a-d	4.6 a	4.2 b-d	3.9 e-g						
BGS	20.3 a-c	16.3 d-h	13.0 g-j	31.9 b	18.8 fg	17.3 g	7.5 a	5.8 b-e	4.3 e-g	4.2 cd	3.6 ij	3.4 lm						
InZEO	22.0 a	19.7 a-e	16.3 d-h	42.2 a	30.6 b	27.4 b-d	7.9 a	7.7 a	7.1 ab	4.6 a	4.2 cd	3.9 fg						
ZEO	20.0 a-d	15.7 f-j	12.7 h-j	31.2 b	18.9 fg	17.2 g	7.5 a	5.7 b-e	4.3 e-g	4.2 d	3.6 ij	3.4 m						
LSD value	3.7682			4.770			1.4881			0.1099								

23.86 and 44.10%), total biomass (significantly, up to 41.1 and 46.45%), 100-grain weight (significantly, up to 14.94 and 22.17%), straw yield (significantly, up to 51.15 and 61.67%), number of spikelets (significantly, up to 23.74 and 38.99%), and number of tillers (up to 23.09 and 46.19%) compared to those in the non-saline condition (1.53 dS m^{-1}), respectively.

In the non-saline condition (1.53 dS m^{-1}), compost-based inoculation increased the plant height (up to 8.14 and 4.69%), root length (up to 13.46 and 4.72%), root weight (up to 7.02 and 3.10%), total biomass (up to 39.73 and 8.76%), 100-grain weight (significantly, up to 11.17 and 7.96%), straw yield (up to 46 and 14.72%), number of spikelets (up to 15.25 and 6.28%), and number of tillers (up to 38.46 and 20%) compared to those in the un-inoculated control and with the liquid inoculum, respectively.

At the salinity of 10 dS m^{-1} , biogas slurry-based inoculation significantly increased the plant height by up to 38.62 and 14.16% compared to that in the un-inoculated control and with the liquid inoculum, respectively, while compost-based inoculation increased the spike length (up to 19 and 7.5%), root length (up to 51.52 and 13.63%), root weight (up to 37.03 and 13.24%), total biomass (significantly, up to 68.96 and 28.26%), 100-grain weight (significantly, up to 19.91 and 11.87%), straw yield (up to 45.85 and 19.99%), and number of spikelets (up to 35.56 and 17.31%) compared to those in the un-inoculated control and with the liquid inoculum, respectively. Both the compost- and biogas slurry-based inoculations increased the number of tillers by up to 40 and 27.25% compared to that in the un-inoculated control and with the liquid inoculum, respectively.

At the salinity of 15 dS m^{-1} , compost-based inoculation also increased the plant height (significantly, up to 34.46 and 14.42%), spike length (up to 18.64 and 6.06%), root length (up to 51.52 and 20.54%), root weight (up to 74.68 and 24.82%), total biomass (significantly, up to 70.57 and 26.74%), 100-grain weight (significantly, up to 23.76 and 8.13%), straw yield (up to 51.4 and 21.96%), and number of spikelets (up to 41.67 and 21.43%) compared to that in the un-inoculated control and with the liquid inoculum, respectively. Both the compost- and biogas slurry-based inoculations also increased the number of tillers per plant by up to 71.43 and 20.12% compared to that in the un-inoculated control and with the liquid inoculum, respectively.

4. Discussion

Saline conditions are known to inhibit the growth and yield of wheat. Reductions in the growth and yield of wheat due to high salinity may be due to the cumulative effects of disruptions to the membrane stability, relative water content, photosynthetic pigment content, and nutrient and hormonal imbalances in the plant (Costa et al., 2018; Talaat and Shawky, 2014). Microbial inoculation has been shown to be an advanced and natural alternative means of inducing salinity tolerance in wheat (Khan et al., 2017). The use of bio-fertilizers can also be enhanced by improving their reliability (Mishra and Dadhich, 2010; Majeed and Muhammad, 2019). Carrier-based bio-formulations provide suitable micro-environments that increase the survival of the bacteria applied in bio-fertilizers (Hassan and Bano, 2015).

The inoculation of soils with plant growth-promoting rhizobacteria has a long history of irregular and unacceptable results. The inefficiency of bacterial inoculants is the reason for the general failure of bio-fertilizers to become established in the soil (Pacheco-Aguirre et al., 2017). Shahzad et al. (2017) also reported that liquid inoculants frequently showed poorer results than carrier-based formulations. One reason for this might be the poorer survival of microbes in broth compared to in carrier-based bio-fertilizers because carrier materials offer some protection to

microbes under unfavorable soil conditions (Khandare et al., 2019; Wang et al., 2015). In the present study, compost, biogas slurry, and zeolite performed well as carriers for multi-strain inoculations, and better promoted the survival of the multi-strain inoculum and improved the growth of wheat under saline conditions. Espiritu (2011) reported that the total biomass produced was increased by using a compost-based inoculant. The satisfactory microbe survival and increased plant growth in carrier-based inoculations might be due to the more suitable micro-environment for the PGPR and their activities provided by the carrier materials.

Multi-strain consortia in compost and biogas slurry performed the best in this study with respect to bacterial survival and achieving maximal improvements to wheat growth. Compost and biogas slurry are organic materials that are rich in plant nutrients and possess the physicochemical properties of ideal carriers (El-Fattah et al., 2013; Khandare et al., 2019) and provide better environments for the survival of the inoculated microbes, ultimately leading to more uptake of nutrients and better growth by the plants (Bashan et al., 2014; Mohammadi et al., 2011; Verma et al., 2010). The application of compost and biogas slurry can increase the amount of organic matter in the vicinity of the roots, and during microbial decomposition of this organic material (compost, biogas slurry) organic acids are released, which may increase nutrient solubilization at specific microsites (Ma, 2019; Prays et al., 2018). Organic carriers also serve as organic substrates that can maintain larger populations of bacteria in the rhizosphere, which might result in the better development of plant roots by improving nutrient availability and ACC deaminase activity (Shahzad et al., 2008). The use of compost as a substrate could be the best alternative to peat for maintaining larger populations of PGPR (Albareda et al., 2008). In this study, compost and biogas slurry carriers with low C:N ratios (15.87 and 8.63, respectively) were used. The presence of organic carriers with low C:N ratios in the rhizosphere can provide a better niche for microbial population growth and activities (Zahir et al., 2007).

In comparison to the other organic carriers tested, crushed corn cob was found to have a higher C:N ratio. Crushed corn cob did not show significantly better results than treatment with the liquid inoculum in this study. The possible reasons for the failure of crushed corn cob as a carrier might have been its relatively high carbon content (Gunjal et al., 2012), production of toxic chemicals that ultimately may have caused the rapid death of microbial cells, and its poor adherence in the seed-coating process. For use in seed-coating, a carrier material should be in powdered form and have good adhering properties (Malusá et al., 2012). In most cases, the inoculated zeolite showed similar results to the inoculated compost and biogas slurry in this study. This fair performance of zeolite might have been due to its properties as a natural absorbent. Zeolite is a natural absorbent and plays a role in the proper immobilization of the bio-inoculant (Pandey and Maheshwari, 2007). In addition to this, zeolite provides a favorable environment to the inoculated microbes, providing them with protection, preservation, and the ability to perform their metabolic activities. Zeolite-based immobilization is non-toxic to microbes, simple, and cost-effective, so zeolite may represent a successful carrier for PGPR inoculation (Putri et al., 2010; Berninger et al., 2017).

Despite the fact that the water holding capacity of the crushed corn cob was higher than that of the other carriers, it had a much higher C:N ratio than those of compost and biogas slurry, and very poor adhesion to seeds compared to compost, biogas slurry, and zeolite. Having a nearly neutral pH and good adhesion to seeds are both important characteristics of a carrier material for use in successful carrier-based inoculation (Malusá et al., 2012). Among the naturally available carrier materials, none can have all of the properties of an ideal carrier, but a good one should have as many as possible.

Differences among carriers with respect to microbial survival might be due to differences in their physicochemical properties (Ma, 2019). The results of this experiment indicated that compost- and biogas slurry-based carriers are capable of allowing PGPR to attain larger populations after 90 days than the other tested carriers, which might be attributed to their low C:N ratio, high water holding capacity, and pH close to 7, which are all key characteristics of good carriers (Sahu and Brahmaprakash, 2016). Both compost and biogas slurry also had a relatively high nitrogen content, to which the greater survival of microbes within them may also be attributed. The viable cell counts in the carriers decreased over the course of 90 days, which may have occurred due to an eventual lack of moisture and nutrients due to bacterial activity and storage conditions (Phiomtan et al., 2013). In comparison to crushed corn cob, relatively large populations of microbes were attained with zeolite, which might be attributed to zeolite being nontoxic and slowly releasing the carried fertilizers into the soil (Yuvaraj and Subramanian, 2018).

The carrier-based inoculation of a multi-strain microbial consortium has several advantages over other approaches, such as the protection of microbes from stressful conditions, a longer inoculum shelf life, and better establishment and survival of the consortium on the seed/root. The physicochemical properties of carriers have important impacts on the survival of the inoculant. The ideal characteristics of carrier material include having a large surface area, being rich in organic matter, having a high water holding capacity and a near-neutral pH, and being easily available and inexpensive. The factors that affect the longevity of the cells of bio-inoculants include temperature, moisture, carrier material, etc. The optimum moisture level and temperature for the maximum survival of the cells in carrier-based inoculants for longer periods of storage are 35 to 50% and 30 °C, respectively (Sangeetha, 2012). It has also been reported that multi-strain consortia carried in composted materials showed more enzymatic activities, such as chitinase activity (Wang et al., 2016).

It was observed in this study that the carrier-based inoculation of bacterial consortia significantly promoted the grain yield, total biomass, and other growth parameters of wheat at different levels of salinity under pot conditions. It was shown that plants in inoculated soil performed better under salinity stress than those in normal soil. These results are also supported by the findings of Príncipe et al. (2007), who showed that inoculated plants grown in saline conditions produced shoots with greater dry weights than those of un-inoculated control plants. It has also previously been reported that different bacterial strains showed different responses to salt-stressed conditions. Multi-strain inoculation played a key role in improving shoot and root growth compared to that of un-inoculated control plants. This increased growth might be due to the lowering of endogenous inhibitory level of ethylene in the roots because of the ACC deaminase activity of PGPR, which ultimately affects shoot growth positively (Zahir et al., 2011). In addition to ACC deaminase activity, this difference in shoot/root growth might be due to other growth-promoting characteristics of the PGPR, such as nitrogen fixation, phosphorus solubilization, phytohormone synthesis, iron chelation, exopolysaccharide (EPS) production, siderophore production, and pathogen inhibition through the synthesis of chitinase and antibiotics (Nadeem et al., 2010b; Rajput et al., 2013; Rana et al., 2012). Ahmad et al. (2013) also stated that PGPR that contained ACC deaminase promoted plant growth and significantly increased root length under pot conditions.

High salinity causes leaf chlorosis in wheat. The reduction in chlorophyll content under high salinity could be due to the suppression of chloroplast biosynthesis, chlorophyllase activity (Talaat and Shawky, 2014), and/or reduced uptake of the minerals required for chlorophyll synthesis (Murkute et al., 2006). It was

also observed in this study that bio-inoculation increased the photosynthetic pigment content (chlorophyll *a* and *b*, and carotenoids) of wheat. Under salinity stress, accelerated ethylene production is known to occur and cause senescence (Arshad and Frankenberger Jr., 2002), so it is very likely that the bio-inoculants inhibited ethylene production, which protected chlorophyll from degradation. The increased chlorophyll content in inoculated plants may also have been due to there being an increased photosynthetic leaf area in inoculated plants compared to that in un-inoculated control plants in which the leaf area was reduced due to salinity stress (Costa et al., 2018).

It was observed that soil salinity caused considerable reductions in the net photosynthetic rate (*A*), transpiration rate (*E*), and stomatal conductance (g_s) in wheat leaves, while the intrinsic CO₂ concentration (*C_i*) was increased by salt stress. This may have been due to the degradation of chlorophyll pigments, reductions in membrane stability and relative water content, and/or nutrient and hormonal imbalances (Talaat and Shawky, 2014). Salt stress disturbs the electrical potential of the plasma membrane, which ultimately causes nutrient deficiencies and water stress. When plants suffer from physiological drought stress due to high salinity, then their stomatal conductance is reduced. A reduced intrinsic CO₂ concentration (*C_i*) and plant water use efficiency (*A/E*) are associated with reduced plant stomatal conductance (g_s). However, the use of bio-inoculants improved these disturbed conditions by preventing chlorophyll from degrading and improving water use efficiency (WUE) and nutrient use efficiency (NUE). Their high intrinsic water use efficiency (*A/g_s*) might have contributed to the enhanced salt tolerance of the inoculated plants (Kanwal et al., 2011; Talaat and Shawky, 2014).

The physiological role of proline in plants is controversial. In this study, the proline content was increased by salinity stress. A plant's proline content may be an indication of its stress status (Rai et al., 2003). In stressful environments, plants increased the content of solutes in their cells, such as proline, which acts as a compatible solute for intercellular osmotic adjustment (Silveira et al., 2003). Moreover, compatible solute accumulation is an energy-consuming process that adds to the already increased metabolic costs of dealing with salinity stress. However, it was found herein that proline concentrations significantly decreased when salt-stressed plants were inoculated with PGPR with ACC deaminase activity. Nadeem et al. (2010a) also found that the proline content decreased in inoculated plants relative to that in un-inoculated plants. This might be attributed to the fact that the multi-strain bacterial inoculation mitigated the severity of the salinity stress on wheat, and thus, ultimately, proline concentrations (a sign of stress) were also reduced (Han and Lee, 2005).

Bio-inoculations not only alleviated the inhibitory effects of salinity on wheat growth, but also induced a marked and progressive increase in crude protein concentrations. High concentrations of ethylene are known to inhibit protein synthesis, while the PGPR used in this study suppressed ethylene production due to their ACC deaminase activity. Similarly, Bharti and Barnawal (2019) also reported an increased protein content after the inoculation of plants with ACC deaminase-containing microbes.

Salinity causes nutritional imbalance, which is one of the main damages done to plants by high salinity. Salinity stress creates an ionic imbalance in plants due to there being more uptake of Na⁺ and less uptake of K⁺, which reduces plant height, leaf surface area, and the activities of major metabolic pathways, leading to plant death in severe conditions (Nadeem et al., 2013b), whereas plants require higher K⁺ than Na⁺ concentrations for their normal metabolic reactions (Nadeem et al., 2013a). Therefore, the K⁺/Na⁺ ratio in plant tissues can be used to examine salt tolerance/salt adaptability in plants. A high K⁺/Na⁺ ratio indicates that a plant is salt tolerant (Abbas et al., 2013). In saline conditions, Na⁺ uptake

effectively competes with K^+ uptake through these ions using common transport systems. Many scientists have found that an increase in the soil Na^+ content caused there to be more uptake of Na^+ and less uptake of K^+ by plants, which resulted in a low K^+/Na^+ ratio in the plant, and as a result the plant may suffer from the toxic accumulation of Na^+ (Cheng et al., 2015). However, in this study, the multi-strain inoculation of plants with PGPR significantly increased the K^+/Na^+ ratio in wheat. A higher K^+/Na^+ ratio is a good indicator of salt tolerance (Shabala and Pottosin, 2014), which may be achieved by plants increasing their uptake of K^+ and/or decreasing (or restricting) their uptake of Na^+ (Yue et al., 2007). Moreover, the reduced concentration of Na^+ in inoculated plants may also be attributed to the production of exopolysaccharides (EPSs) by the inoculated bacteria. These EPS-producing bacteria have the ability to restrict Na^+ uptake by plant roots by binding these and other cations (Ashraf et al., 2004; Gupta and Diwan, 2017). The greater uptake of nutrients in inoculated plants may have been the reason these crops performed better under saline stress than un-inoculated plants.

Inoculation also resulted in a significant increase in root growth compared to that in un-inoculated control plants. It is very likely that the bio-inoculant improved the growth of roots by lowering the endogenous inhibitory level of ethylene in them, which is a negative regulator of root growth. Similarly, Shahzad et al. (2010) and Zahir et al. (2011) reported that PGPR regulated ethylene biosynthesis, which subsequently affected growth. However, the efficiency of the liquid inoculum was greatly influenced by the different carrier materials with which it was applied.

5. Conclusion

In this study, it was concluded that bio-inoculation can improve the growth, physiological, and biochemical parameters of wheat, and that the use of carrier materials can further enhance these parameters in the plant. Compost and biogas slurry seemed to be the locally available carriers that were the best able to enhance the efficacy of multi-strain bacterial consortia to promote plant growth under saline stress, most likely due to these carrier materials providing the best micro-environment for the survival and physiological activities of PGPR. This study paved the way for the use of compost, biogas slurry, and zeolite as carriers to be evaluated more in further studies to improve the performance of bio-inoculants in improving the growth and yield of wheat under saline conditions.

Declaration of Competing Interest

The authors declared that there is no conflict of interest.

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