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BACILLUS AMYLOLIQUEFACIENS AND *ALCALIGENES FAECALIS* WITH BIOGAS SLURRY IMPROVED MAIZE GROWTH AND YIELD IN SALINE-SODIC FIELD

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Abstract

Abiotic stresses are among the major threats in agriculture to achieve optimum growth and yield of crops. Besides other abiotic stresses, salinity and sodicity problems are centre of attention. Higher uptake of undesirable salts, hamper the growth of plants. These salts increase the production of stress ethylene, exerting negative effects on growth. In addition, imbalance in nutrients uptake drastically decrease the yield of crops. To overcome the problem of salt stress, use of plant growth promoting rhizobacteria (PGPR) is an efficacious technique. However, to increase the efficacy of this technique, use of organic amendments with PGPR is mostly suggested. Therefore, the current study was conducted to examine the role of ACC deaminase producing rhizobacterial strains i.e. *Bacillus amyloliquefaciens* and *Alcaligenes faecalis* in separate and conjoint with biogas slurry (BGS) on growth and yield of maize in saline-sodic field conditions. Recommended Nitrogen (N), phosphorus (P) and potassium (K) fertilizers (NPK) were applied to fulfill crop nutrients requirement. Application of *Alcaligenes faecalis*+BGS+NPK gave maximum increase in plant height (16%), number of grain cob^{-1} (17%), 1000-grain weight (16%), total chlorophyll content (11%), carotenoids (1.9 fold), anthocyanin (2.3 fold), shoot P (32%), shoot K (15%), grain N (23%), grain P (51%), grain K (27%), grain yield (51%) and straw yield (66%), over control. In conclusion, *A. faecalis* proved more effective as compared to the *B. amyloliquefaciens.* Both the PGPR strains, *B. amyloliquefaciens* and *A. faecalis* have potential to improve maize growth and yield when applied in combination with BGS under saline sodic condition.

Key words: Rhizobacteria, Corn, Plant nutrition, Bio-fertilizer, Photosynthetic pigments, Organic amendment.

Introduction

Imbalanced or limited bioavailability of nutrients to crops, is a major hurdle to achieve the goal of producing sufficient food for human beings, around the globe (Fageria & Baligar, 2005). In less fertile soils, limited availability of nitrogen (N), phosphorus (P) and potassium (K) enhanced the synthesis of stress ethylene which induced adverse effects on crops growth and productivity (Glick *et al.*, 1998; Khan *et al*., 2015). Moreover, higher concentration of exchangeable and soluble salts in soils also decrease crops yield through biochemical and physiological changes in plants (Hussain *et al*., 2013; Iqbal *et al*., 2014). Salt-affected soil causes decrease in crop yield up to 50% (Eynard *et al*., 2005). Soil sodicity and salinity problems may be solved by leaching salts out of rhizosphere or through gypsum addition to the soil in separate or with organic amendments (Horneck *et al*., 2007).

For sustainable and economical production of crops under low soil fertility, the conjoint use of plant growth promoting rhizobacteria (PGPR) and inorganic fertilizers is commonly suggested (Vessey, 2003; Zafar-ul-Hye *et al*., 2015; Yaseen *et al*., 2019). The PGPR play an imperative role in essential ecosystem processes i.e., seedling establishment, nutrient recycling and controlling plant pathogens (Glick, 1995; Elo *et al*., 2000; Weller *et al*., 2002; Danish *et al*., 2019; Danish *et al*., 2020). For saline-sodic soil condition, PGPR may be much helpful for plant growth (Zahir *et al*., 2009) along with mineral fertilization (Hussain *et al*., 2017). Under stress conditions, higher production of 1-aminocyclopropane-1-carboxylic acid (ACC) and consequently ethylene, soon after sowing inhibit root elongation (Alarcon *et al*., 2009; Danish & Zafar-ul-Hye, 2019), which ultimately leads to significant damage to the plants (Grichko & Glick, 2001; Schallet, 2012). As a result of use of ACC deaminase positive PGPR, ethylene level is decreased resulting in growth improvement (Bhattacharyya & Jha, 2012; Glick, 2014). On the other hand, secretions of phytohormones and enzymes by PGPR during root colonization significantly enhance the growth of host plants (Egamberdiyeva, 2007; Lugtenberg & Kamilova, 2009; Ogbo, 2012). So the role of PGPR has been established for improving plant growth, yield and nutrient uptake (Wu *et al*., 2005; Aon *et al*., 2015).

Biogas slurry (BGS) is obtained during the production of biogas using livestock manure as waste biomass which is a rich source of macro and micronutrients (Islam *et al*., 2010). To improve the fertility status of soil, scientists have suggested to use BGS as an eco-friendly organic amendment (Islam *et al*., 2010; Sieling *et al*., 2013). Application of BGS in soil significantly enhances soil organic matter due to the presence of large amount of organic carbon in manures (Urselmans *et al*., 2009).

On account of exhaustive crop nature, the consumption of nutrients is quite high in maize (*Zea mays* L.) which significantly enhances the production cost (Cassman *et al*., 1998; Errebhi *et al*., 1999). Its 70% cultivation is to fulfill the food requirement as a forage and 30% cultivation is for grain use (Rosegrant *et al*., 2009). Maize crop is highly sensitive to salt-stress (Parvaiz, 2014; Farooq *et al*., 2015, Zafar-ul-Hye *et al*., 2015). It has been reported that PGPR improve growth, yield and physiological attributes of maize under saline conditions (Nadeem *et al*., 2006; Zafar-ul-Hye *et al*., 2018). However, a limited literature is available on the combined application of PGPR and BGS to enhance the maize productivity on saline-sodic fields. Therefore, keeping in mind the importance of maize as 3rd largest cultivated cereal crop (Danish *et al.*, 2015), a field study was conducted with the hypothesis that seed inoculation with rhizobacterial strains i.e *Bacillus amyloliquefaciens* and/or *Alcaligenes faecalis* with BGS and NPK fertilizers would improve the growth and yield of maize on saline-sodic soil.

Materials and Methods

Preparation of inocula: Two ACC-deaminase producing PGPR strains, *Bacillus amyloliquefaciens* and *Alcaligenes faecalis* were taken from the Laboratory of Soil Microbiology and Biochemistry, Department of Soil Science, Bahauddin Zakariya University.

The concentration of indole acetic acid (IAA) was determined by using Salkowski reagent on a spectrophotometer at 535 nm wavelength with and without L-tryptophan (Sarwar *et al.*, 1992). ACC-deaminase activity was estimated according to Jacobson *et al.*, (1994). The phosphate solubilization activity of rhizobacterial strains was examined using Agar medium having tri-calcium phosphate as an inorganic form of phosphorus (Goldstein, 1986).

For preparation of inocula, DF broth without agar having ACC as a sole source of nitrogen (Dworkin & Foster, 1958) was used. Finally, optical density (OD) of inocula was maintained to 0.45 at 540 nm to achieve uniform population (10^7-10^8 cfu mL^{-1}) as described by Ahmad *et al.*, (2011). The characteristics of both the PGPR strains are given in Table 1.

Seeds sterilization and inoculation: Maize hybrid (Kenzo-123 hybrid) seeds were used in the current study. Before inoculation with the PGPR, the seeds were sterilized. For inoculation of 100 g seeds, 1ml of inocula with 10% sugar solution was added in a sterilized beaker. Finally, top dressing of seeds was done with sterilized peat and clay (3:1) mixture (Ahmad *et al.*, 2015).

Collection and characterization of biogas slurry (BGS): The BGS was collected from a locally installed biogas plant. For characterization, the BGS was initially air-dried and then digested with di-acid mixture (HNO_3:$HClO_4$) for the analysis of total P (Jones *et al.*, 1991) and total K (Nadeem *et al.*, 2013) on spectrophotometer and flamephotometer, respectively. For the determination of total N, the BSG was digested with sulfuric acid as described by Jones *et al.*, (1991). The chemical characteristics of BSG are given in Table 1.

Experimental plan and layout: In the experimental area of the Department of Soil Science, 30 plots were well prepared following standard cultural practice (3-4 times intercrossing harrowing followed by planking with each plough). Recommended N, P and (NPK) fertilizers were applied at the rate of 260, 225, 150 kg ha^{-1}, respectively. The fertilizers i.e. urea, diammonium phosphate (DAP) and muriate of potash (MOP) were used as a source of N, P and K, respectively. The DAP and SOP were incorporated in a single dose on the sowing time, while, urea was applied in three split doses at 1st, 3rd and 5th irrigation. In a randomized complete block design (RCBD), ten treatments i.e., control, NPK (Recommended N, P and K fertilizer dose), *Bacillus amyloliquefaciens*, *Alcaligenes faecalis*, BGS (BGS at the rate of 6 Mg ha^{-1}), *Bacillus amyloliquefaciens* +NPK, *Alcaligenes faecalis* + NPK, BGS + NPK, *Bacillus amyloliquefaciens* + BGS + NPK and *Alcaligenes faecalis* + BGS + NPK were used with three replications.

Crop husbandry: According to the treatment plan, seeds (either un-inoculated or inoculated with *Bacillus amyloliquefaciens* or *Alcaligenes faecalis*) were sown at the rate of 20 kg ha^{-1} by dibble method. The row to row distance was 75 cm while plant to plant distance was maintained at 20-25 cm. Recommended agronomic practices were followed (A hand hoeing about 25 days after sowing provides satisfactory weed control at small scale) to raise the healthy crop.

Table 1. Pre-experiment characterization of soil, biogas slurry and PGPR strains *Bacillus amyloliquefaciens* and *Alcaligenes faecalis*.

Soil	Unit	Value*	Biogass slurry	Unit	Value
Sand	%	51.5	pH_s	-	7.12
Silt	%	25.2	EC_e	dS m^{-1}	3.01
Clay	%	23.3	Organic C	%	48.0
Texture	**Sandy clay loam**		C:N	-	30.4
pH_s	-	8.22	Total N	%	1.58
EC_e	dS m^{-1}	5.24	Total P	%	1.70
CEC	$cmol_c$ kg^{-1}	5.21	Total K	%	1.31
Total N	**%**	**0.03**	**PGPR**	***Bacillus amyloliquefaciens***	***Alcaligenes faecalis***
Extractable P	mg kg^{-1}	7.39	P-solubilization	+	+
Extractable K	mg kg^{-1}	107	ACC deaminase	+	+
SAR	-	14.5	Indoleacetic acid	+	+
ESP	%	17.8			

+ Represents that activity is present

Harvesting: For the determination of photosynthetic (chlorophyll a, b and total) and accessory pigments (i.e. carotenoids and anthocyanin) in maize leaves, plant samples were collected after 7 weeks of sowing. For growth and yield related parameters at maturity stage, plant height and cob length were measured using scale. For 1000-grain weight analytical balance was used. The number of grains per row and number of grain rows cob^{-1} were manually counted.

Photosynthetic and accessory pigments: For the determination of photosynthetic pigments (chlorophyll a, b and total) in maize leaves, extract was taken using 80% acetone (10 ml). Optical density (OD) of the extract was found out on 663, 645, 530, 503, and 480 nm wavelengths on spectrophotometer (Arnon, 1949). Final calculations were made using the following equations:

$$\text{Chlorophyll a (mg g}^{-1}\text{ f.wt.)} = \frac{12.7\ (\text{OD } 663) - 2.69\ (\text{OD } 645) \times \text{V}}{1000\ \times \text{W}}$$

$$\text{Chlorophyll b (mg g}^{-1}\text{f.wt.)} = \frac{(22.9\ \times \text{OD } 645) + (8.02\ \times \text{OD } 663) \times \text{V}}{1000\ \times \text{W}}$$

$$\text{Total Chlorophyll (mg g}^{-1}\text{ f.wt.)} = \frac{20.2\ (\text{OD } 645) - 4.68\ (\text{OD } 663) \times \text{V}}{1000\ \times \text{W}}$$

$$\text{Carotenoids (mg g}^{-1}\text{ f.wt.)} = \frac{\text{OD}480 + (0.1144 \times \text{OD}663) - (0.638\ \times \text{OD } 645) \times \text{V}}{1000\ \times \text{W}}$$

$$\text{Anthocyanin } (\mu\text{mol ml}^{-1}) = (0.8173 \times \text{OD } 537) - (0.00697 \times \text{OD } 645) - (0.002228 \times \text{OD } 663)$$

where, f.wt. = Fresh Weight, V = final volume made and W = grams of fresh leaf sample

Chemical Analyses of maize shoot and grains: For the determination of N, P and K in maize shoot and grain, samples were digested. Di-acid digestion of maize shoot and grains was done for the analysis of P (Jones *et al*., 1991) and K (Nadeem *et al.,* 2013). However, for the analysis of nitrogen through distillation on Kjeldahl's distillation apparatus (Van Schouwenberg & Walinge, 1973), the samples were digested with H_2SO_4 and digestion mixture (Jones *et al*., 1991).

Yield attributes: For the determination of harvest index and biological yield, following relations were used (Imran *et al*., 2016):

$$\text{Harvest Index (\%)} = \frac{\text{Grain Yield}}{\text{Biological Yield}} \times 100$$

Biological yield (Mg ha^{-1}) = No. of plnats rows – Row distance x Row length x No. of rows x 100

Statistical analysis

Data and standard deviation were computed on Microsoft Excel 2013® software (Microsoft Corporation, Redmond, WA-USA) and to test the significance of treatments, statistical software, Statistix 8.1® was used. Significantly different treatment means were separated by Tukey-HSD test at $p \leq 0.05$ (Steel *et al*., 1997).

Results

Maize growth attributes: The treatments NPK, BGS, and strain *Bacillus amyloliquefaciens* and *Alcaligenes faecalis* inoculation showed significantly variable effect on maize growth attributes. As a result of NPK fertilizers application, a significant increment in number of grains cob^{-1} (30%) and 1000-grain weight (22%) was observed, over control treatment. The treatment *Alcaligenes faecalis* + NPK + BGS resulted in maximum increase in plant height (16%), number of grains cob^{-1} (20%) and 1000-grain weight (16%), over NPK treatment. In the case of cob length, only the treatments *Alcaligenes faecalis* + NPK + BGS and *Bacillus amyloliquefaciens* + NPK + BGS showed a significant increase of 37 and 35%, respectively, over the control. However, this increase was statistically at par with the cob length obtained from NPK treatment (Table 2).

Photosynthetic and accessory pigments: Effect of NPK, BGS, and strains, *Bacillus amyloliquefaciens* and *Alcaligenes faecalis* inoculation on various maize photosynthetic and accessory pigments is shown in Table 3. Application of recommended dose of NPK fertilizers did not show a significant increase in chlorophyll a and b contents in maize tissue, over control. However, as compared to the control, a significant improvement of 13% in total chlorophyll content was observed through NPK addition (Table 3). As compared to the control, S *Alcaligenes faecalis* + NPK + BGS resulted in maximum increase (9%) in chlorophyll a content, followed by the treatment *Bacillus amyloliquefaciens* + NPK + BGS with 7% greater chlorophyll a content. The *Alcaligenes faecalis* + NPK + BGS resulted in maximum increase of 57% in chlorophyll b content, over control. The *Alcaligenes faecalis* + NPK + BGS and *Bacillus amyloliquefaciens* + NPK + BGS resulted in a respective increase of 11 and 9% in total chlorophyll content, over control. As compared to the control, NPK addition did not show a significant increase in the carotenoid and anthocyanin content. The treatments, *Alcaligenes faecalis* + NPK + BGS and *Bacillus amyloliquefaciens* + NPK + BGS resulted in maximum increase of 95 and 89%, respectively, in carotenoid content of maize, over NPK treatment. The *Alcaligenes faecalis* + NPK + BGS and *Bacillus amyloliquefaciens* + NPK + BGS produced 2.3 and 1.9 fold greater anthocyanin content, respectively, over control (Table 3).

Table 2. Effect of PGPR strains (*Bacillus amyloliquefaciens* and *Alcaligenes faecalis*), recommended NPK fertilizer dose and biogas slurry on maize plant height, cob length, grains rows cob^{-1}, number of grains cob^{-1} and 1000-grain weight.

Treatment	Plant height (cm)	Cob length (cm)	Number of grains Cob^{-1}	1000-Grain weight (g)
Control	156.3 ± 3.8 d	13.3 ± 0.33 c	239.7 ± 6.74 g	267.0 ± 6.35 g
Recommended NPK	167.7 ± 1.5 cd	15.3 ± 0.33 a-c	312.7 ± 4.98 e	325.0 ± 3.79 e
B. amyloliquefaciens	177.3 ± 5.2 bc	16.3 ± 0.88 a-c	334.7 ± 6.49 b-d	340.3 ± 6.94 c-e
A. faecalis	179.7 ± 8.0 a-c	15.7 ± 0.88 a-c	323.3 ± 6.01 c-e	340.3 ± 6.94 c-e
BGS	164.7 ± 2.7 cd	15.0 ± 0.58 bc	287.7 ± 7.51 f	300.7 ± 6.74 f
B. amyloliquefaciens + Recommended NPK	171.3 ± 4.7 cd	16.3 ± 0.33 a-c	337.0 ± 10.1 bc	335.3 ± 7.62 de
A. faecalis + Recommended NPK	188.7 ± 3.5 ab	16.0 ± 0.58 a-c	352.0 ± 7.22 ab	357.0 ± 6.35 bc
BGS + Recommended NPK	176.0 ± 3.2 bc	15.7 ± 0.67 a-c	318.3 ± 3.76 de	346.7 ± 5.78 cd
B. amyloliquefaciens + Recommended NPK + BGS	191.3 ± 4.7 ab	18.0 ± 0.58 ab	347.0 ± 7.09 b	366.3 ± 6.98 ab
A. faecalis + Recommended NPK + BGS	195.0 ± 9.6 a	18.3 ± 0.33 a	365.3 ± 7.26 a	377.3 ± 5.78 a

Means ± standard error of means for three replicates. Same letter(s) for each parameter do not differ significantly based on Tukey-HSD test at $p \leq 0.05$; BGS represents biogas slurry

Table 3. Effect of PGPR strains (*Bacillus amyloliquefaciens* and *Alcaligenes faecalis*), recommended NPK fertilizer dose and biogas slurry on photosynthetic and accessory pigments of maize leaves.

Treatment	Chlorophyll a (mg g^{-1})	Chlorophyll b (mg g^{-1})	Total chlorophyll (mg g^{-1})	Carotenoids (µg g^{-1})	Anthocyanin (µmol ml^{-1})
Control	0.455 ± 0.004 b	0.26 ± 0.016 c	0.71 ± 0.023 d	9.96 ± 0.90 d	0.09 ± 0.003 d
Recommended NPK	0.481 ± 0.007 ab	0.33 ± 0.032 a-c	0.80 ± 0.034 bc	11.2 ± 1.79 d	0.12 ± 0.009 cd
B. amyloliquefaciens	0.488 ± 0.007 a	0.35 ± 0.023 a-c	0.83 ± 0.017 a-c	19.2 ± 0.16 ab	0.14 ± 0.007 bc
A. faecalis	0.482 ± 0.013 ab	0.35 ± 0.028 a-c	0.82 ± 0.022 a-c	17.9 ± 1.07 ab	0.14 ± 0.009 bc
BGS	0.454 ± 0.013 ab	0.30 ± 0.028 bc	0.75 ± 0.028 cd	12.2 ± 1.52 cd	0.11 ± 0.010 cd
B. amyloliquefaciens+Recommended NPK	0.480 ± 0.009 ab	0.34 ± 0.014 a-c	0.82 ± 0.022 a-c	17.6 ± 0.31 ab	0.13 ± 0.008 b-d
A. faecalis+Recommended NPK	0.481 ± 0.006 ab	0.38 ± 0.009 ab	0.86 ± 0.009 a-c	18.7 ± 0.62 ab	0.18 ± 0.011 b
BGS + Recommended NPK	0.488 ± 0.004 a	0.35 ± 0.025 a-c	0.82 ± 0.016 a-c	16.2 ± 0.09 bc	0.13 ± 0.013 b-d
B. amyloliquefaciens+Recommended NPK + BGS	0.489 ± 0.009 a	0.39 ± 0.012 ab	0.87 ± 0.010 ab	21.2 ± 0.98 a	0.23 ± 0.007 a
A. faecalis+Recommended NPK+BGS	0.496 ± 0.004 a	0.41 ± 0.016 a	0.89 ± 0.012 a	21.8 ± 0.81 a	0.28 ± 0.013 a

Means ± standard error of means for three replicates. Same letter(s) for each parameter do not differ significantly based on Tukey-HSD test at $p \leq 0.05$; BGS represents biogas slurry

Table 4. Effect of PGPR strains (*Bacillus amyloliquefaciens* and *Alcaligenes faecalis*), recommended NPK fertilizer dose and biogas slurry on N, P and K concentration in maize shoot and grain.

Treatment	Nitrogen (%)	Phosphorus (%)	Potassium (%)
	Nutrient concentration in maize straw		
Control	0.86 ± 0.04 c	0.21 ± 0.013 g	1.48 ± 0.04 e
Recommended NPK	1.14 ± 0.10 ab	0.25 ± 0.017 d-f	1.65 ± 0.03 b-d
B. amyloliquefaciens	1.04 ± 0.10 a-c	0.22 ± 0.001 fg	1.53 ± 0.02 de
A. faecalis	1.06 ± 0.02 a-c	0.23 ± 0.013 e-g	1.65 ± 0.06 b-d
BGS	0.97 ± 0.08 bc	0.21 ± 0.010 fg	1.57 ± 0.03 c-e
B. amyloliquefaciens + Recommended NPK	1.16 ± 0.03 ab	0.28 ± 0.005 b-d	1.71 ± 0.04 bc
A. faecalis + Recommended NPK	1.13 ± 0.02 ab	0.32 ± 0.007 ab	1.70 ± 0.04 bc
BGS + Recommended NPK	1.26 ± 0.05 a	0.27 ± 0.017 c-e	1.75 ± 0.06 ab
B.amyloliquefaciens + Recommended NPK + BGS	1.22 ± 0.03 ab	0.31 ± 0.006 a-c	1.78 ± 0.06 ab
A. faecalis + Recommended NPK + BGS	1.23 ± 0.04 ab	0.33 ± 0.022 a	1.89 ± 0.03 a
	Nutrient concentration in maize grain		
Control	1.24 ± 0.04 d	0.32 ± 0.01 d	1.34 ± 0.03 e
Recommended NPK	1.44 ± 0.04 c	0.43 ± 0.04 cd	1.35 ± 0.01 e
B. amyloliquefaciens	1.44 ± 0.04 c	0.44 ± 0.04 c	1.42 ± 0.01 de
A. faecalis	1.46 ± 0.03 c	0.44 ± 0.01 c	1.52 ± 0.05 c
BGS	1.41 ± 0.07 cd	0.40 ± 0.01 cd	1.38 ± 0.02 e
B. amyloliquefaciens + Recommended NPK	1.65 ± 0.03 ab	0.48 ± 0.01 c	1.55 ± 0.02 bc
A. faecalis + Recommended NPK	1.65 ± 0.03 ab	0.51 ± 0.05 bc	1.47 ± 0.02 cd
BGS + Recommended NPK	1.54 ± 0.04 bc	0.52 ± 0.04 bc	1.41 ± 0.01 de
B. amyloliquefaciens + Recommended NPK + BGS	1.68 ± 0.05 ab	0.60 ± 0.05 ab	1.63 ± 0.02 b
A. faecalis + Recommended NPK + BGS	1.77 ± 0.03 a	0.65 ± 0.01 a	1.72 ± 0.06 a

Means ± standard error of means for three replicates. Same letter(s) for each parameter do not differ significantly based on Tukey-HSD test at $p \leq 0.05$; BGS represents biogas slurry

Macro-nutrients concentration in shoot and grain: Application of recommended NPK fertilizers showed a significant increase of 33% in shoot N concentration, over control. The BGS + NPK resulted in the maximum increase (47%) in shoot N concentration, over control; however, this increase was statistically comparable with NPK treatment. As compared to the control, a significant increase in shoot P and K concentration (19 and 11%, respectively) was observed as a result of NPK fertilizers addition. The treatment, *Alcaligenes faecalis*+NPK+BGS resulted in maximum increase in shoot P and K (32 and 15%, respectively), over the NPK treatment (Table 3).

Recommended NPK fertilizers showed a significant increase of 16% in grain N concentration, over control. The *Alcaligenes faecalis* + NPK + BGS showed maximum increase (15%) in grain N concentration, over NPK treatment, while, this increase was statistically at par with the results of *Bacillus amyloliquefaciens* + NPK + BGS, BGS + NPK, *Alcaligenes faecalis* + NPK and *Bacillus amyloliquefaciens* + NPK treatments. As compared to the control, NPK fertilizers addition did not show a significant increase in grain P and K concentration. The treatment *Alcaligenes faecalis* + NPK + BGS resulted in the maximum increase in grain P and K concentration (51 and 27%, respectively) over NPK treatment (Table 4).

Harvest index and yield: Effect of treatments was significant for harvest index (Fig. 1), grain yield (Fig. 2), biological yield (Fig. 3) and straw yield (Fig. 4) of maize. However, regarding the yield parameters, there was non-significant difference between the control and NPK treatment (Fig. 1-4). The *Alcaligenes faecalis* + NPK + BGS resulted in maximum increase (33.1%) of harvest index, which was significantly greater (39 and 25%, respectively) than the control and NPK treatment (Fig. 1). As compared to the control, the *Alcaligenes faecalis* + NPK + BGS resulted in the maximum increase (51%) in grain yield, followed by the treatment *Bacillus amyloliquefaciens* + NPK + BGS with 38% increase (Fig. 2). As compared to the NPK treatment, the *Bacillus amyloliquefaciens* + NPK + BGS resulted in the maximum increase (62.3%) in biological yield, followed by the treatment *Bacillus amyloliquefaciens* + NPK with 32% increment (Fig. 3). As compared to the NPK treatment, the *Alcaligenes faecalis* + NPK + BGS gave maximum (with an increase of 66%) straw yield, followed by the treatment *Alcaligenes faecalis* + NPK with 61% increment in straw yield (Fig. 4).

Discussion

The data have revealed that abiotic stresses like salinity and sodicity, markedly suppress plant growth (Wu *et al*., 2015). Under abiotic stress, production of ACC and ethylene is enhanced in roots, which is thought to be a big reason for root growth restriction (Okamoto *et al*., 2008; Dubois *et al*., 2018; Zafar-ul-Hye *et al.,* 2019a). Combined application of PGPR, BGS and NPK remained significantly better as compared to their sole applications for maize growth and yield under saline soil conditions. The improvement in the growth and yield of maize through combined application of PGPR, BGS and NPK might be due to the better uptake of nutrients. The integrated use of inorganic and organic fertilizers along with PGPR is a better approach for significant increase in crop productivity under field conditions (Koushal & Singh, 2011). As BGS contains mineral nutrients in addition to appreciable quantity of organic matter, so this might be a possible reason for improved plant growth response due to changing scenario of soil fertility management.

In stress environments, root growth and seed germination are adversely affected due to high level of ethylene production (Iqbal *et al*., 2017; Dubois *et al*., 2018). In the current scenario, the ACC-deaminase activity of both PGPR strains probably suppressed stress-induced ethylene level. Thus, the immediate precursor of ethylene, ACC might have been converted to ammonia and α-ketobutyrate. Therefore, the reduction in stress ethylene level resulted in an improvement in plant growth. Improvement in plant growth by ACC deaminase producing rhizobacteria might also be due to better nutrients uptake (Biari *et al*., 2008). Seed inoculation with PGPR also increases the efficiency of applied fertilizers (Chattha *et al*., 2017; Zafar-ul-Hye *et al*., 2019b). Moreover, it is also reported that under salt-affected conditions, the crops require more nitrogenous fertilizers than the normal soils (Mehdi *et al*., 2007). In the current study, the integrated use of organic and inorganic fertilizers possibly reduced crop dependence on mineral fertilizers and also resulted in an enhancement of plant growth response. The rhizobacterial strains, *Bacillus amyloliquefaciens* and *Alcaligenes faecalis* were found capable too for producing IAA and phosphate solubilization, which possibly contributed towards growth promotion under saline-sodic field condition.

A significant improvement in the plant height, cob length, number of grains cob^{-1}, grain yield and straw yield also justified the imperative role of combined organic and inorganic fertilizers along with the PGPR strains, *Bacillus amyloliquefaciens* and *Alcaligenes faecalis*. Improved total chlorophyll content in maize tissue, as a result of *Alcaligenes faecalis* + NPK + BGS application, were the indication of significant improvement in plant growth, and straw and grain N concentration. Better uptake of N in plants is one of the solid reasons for the improvement in the chlorophyll content of plant (Hashim *et al*., 2017).

Biogas slurry provides a significant amount of carbon to the soil microorganisms to facilitate the process of mineralization which ultimately results in significant increase of nutrients content (Li, 2002; Kumar *et al*., 2015). Biogas slurry also contains nutrients comparative to organic matter. In the current scenario, BGS possibly played a significant role to modify of soil fertility status, which ultimately resulted in a significant improvement in plant growth and nutrients accumulation. Under saline conditions, demand for N in crops is increased (Mehdi *et al*., 2007). In the present experiment, BGS possibly improved soil nutrient status, as it contained 1.58, 1.70 and 1.31% N, P and K, respectively. It is reported that nutrients from organic sources are more effective than those from chemical sources (Ahmad *et al*., 2014). So, this might be the possible reason of improved plant nutritional status. Less accumulation of ethylene in the roots of plants also enhances the uptake of N, P and K (Zheng *et al*., 2013). The ethylene in the roots of plants diffuses out in the rhizosphere where ACC deaminase cleaves it into ammonia and α-ketobutyrate (Glick *et al*., 1998).

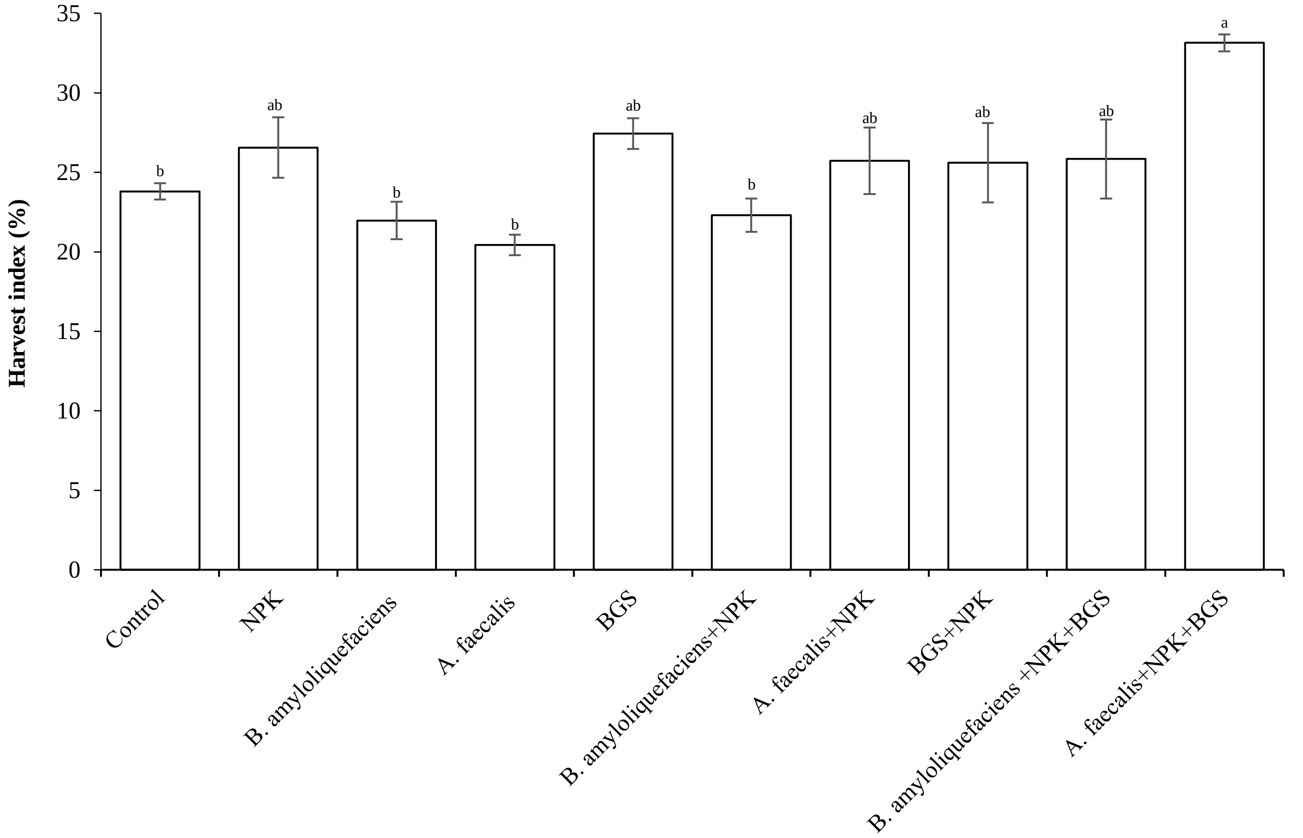

Fig. 1. Effect of PGPR strains, recommended NPK fertilizer dose and biogas slurry on harvest index of maize in saline-sodic field; NPK: Recommended NPK fertilizer dose, BGS: Biogas slurry; Bars are of means and error bars are of standard error of means for three replicates. Same letter(s) for each parameter do not differ significantly based on Tukey-HSD test at $p \leq 0.05$.

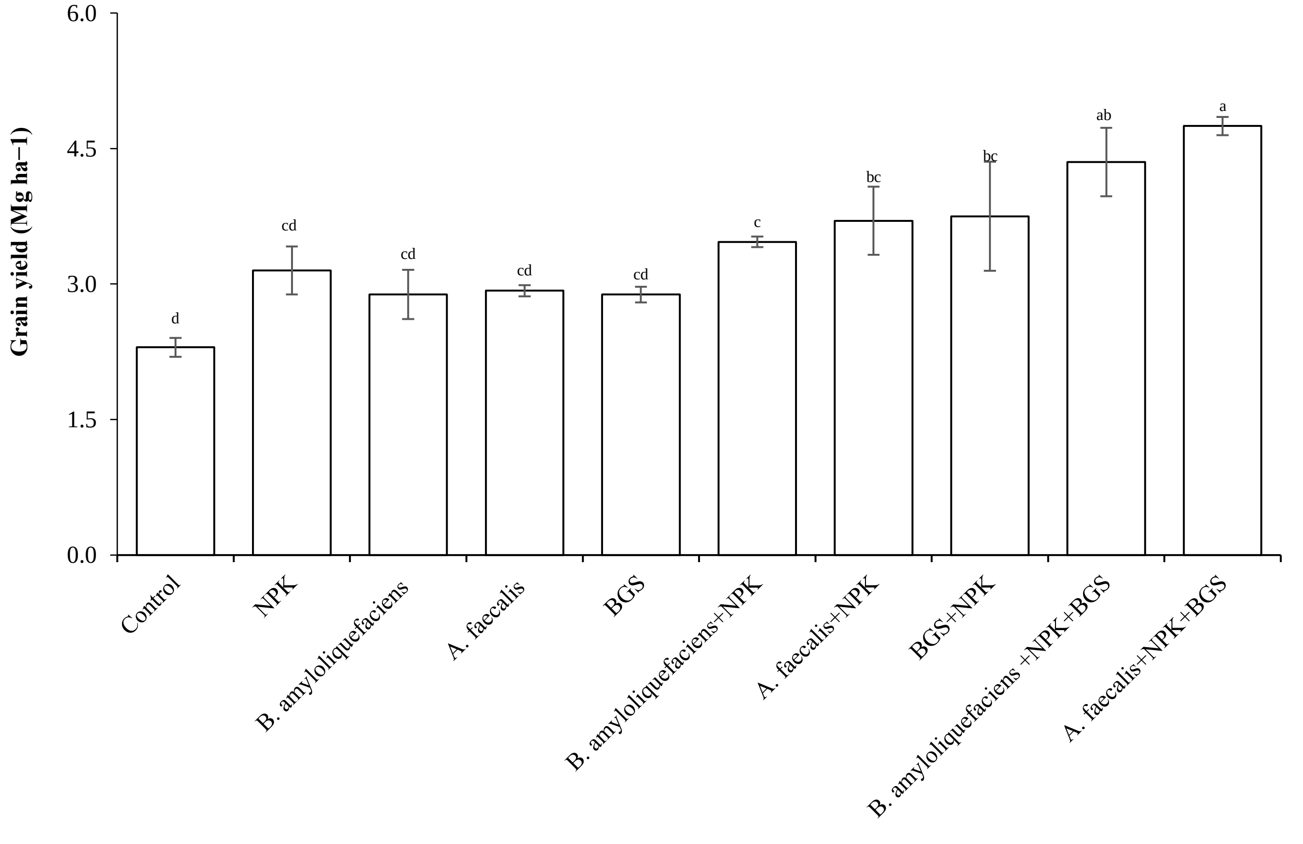

Fig. 2. Effect of PGPR strains, recommended NPK fertilizer dose and biogas slurry on grain yield of maize in saline-sodic field; NPK: NPK: Recommended NPK fertilizer dose, BGS: Biogas slurry; Bars are of means and error bars are of standard error of means for three replicates. Same letter(s) for each parameter do not differ significantly based on Tukey-HSD test at $p \leq 0.05$.

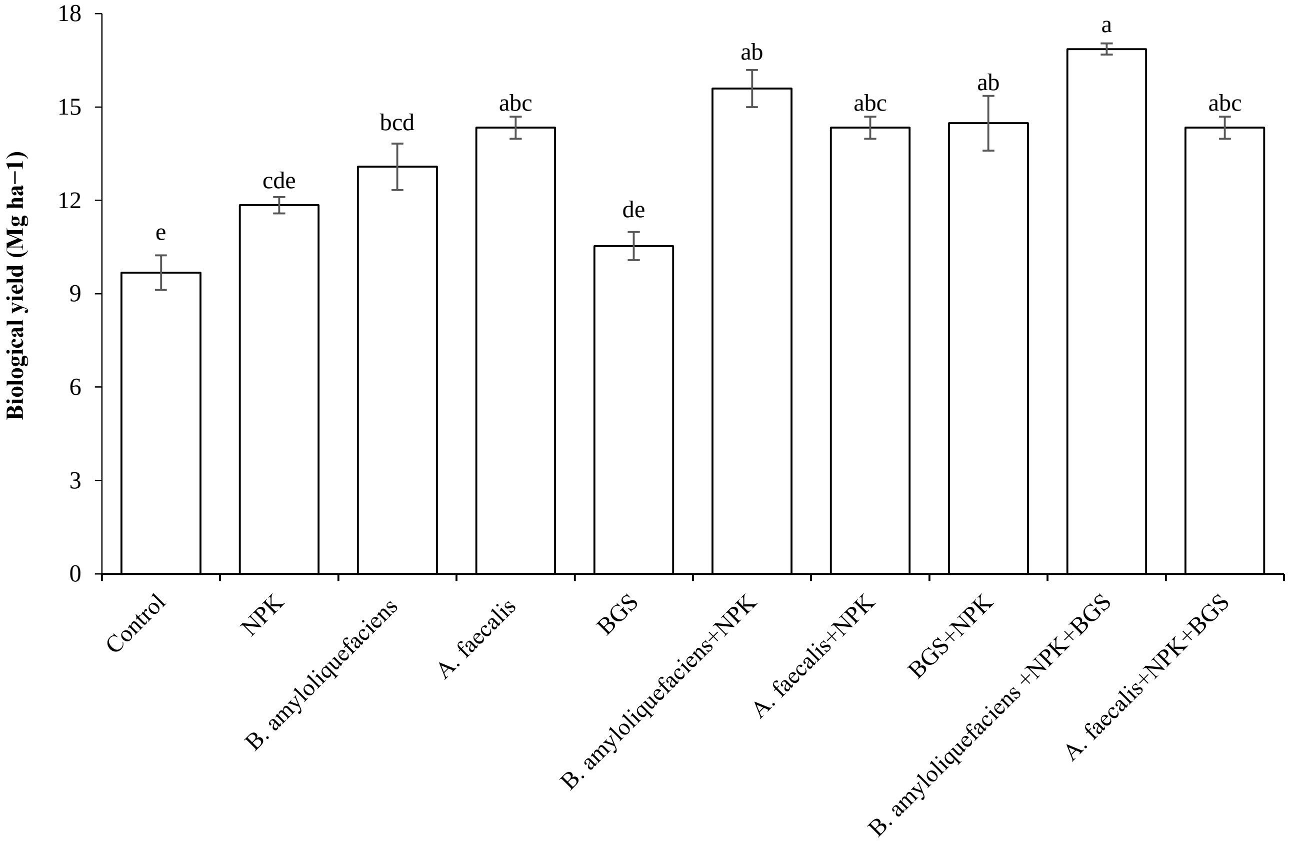

Fig. 3. Effect of PGPR strains, recommended NPK fertilizer dose and biogas slurry on biological yield of maize in saline-sodic field; NPK: NPK: Recommended NPK fertilizer dose, BGS: Biogas slurry; Bars are of means and error bars are of standard error of means for three replicates. Same letter(s) for each parameter do not differ significantly on Tukey-HSD test at $p \leq 0.05$.

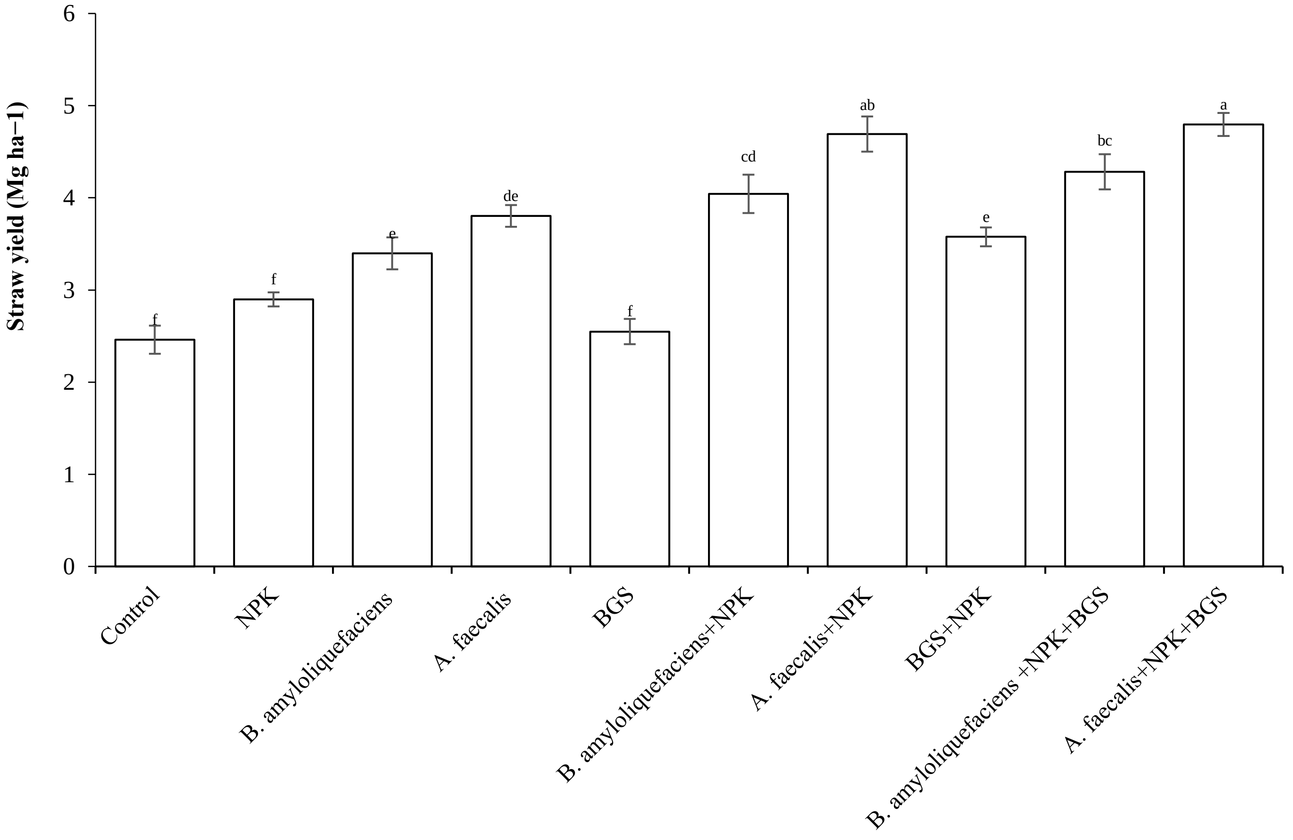

Fig. 4. Effect of PGPR strains, recommended NPK fertilizer dose and biogas slurry on straw yield of maize in saline-sodic field; NPK: NPK: Recommended NPK fertilizer dose, BGS: Biogas slurry; Bars are of means and error bars are of standard error of means for three replicates. Same letter(s) for each parameter do not differ significantly based on Tukey-HSD test at $p \leq 0.05$.

Conclusion

The combined application of *Bacillus amyloliquefaciens* and *Alcaligenes faecalis* and BGS could impart more resistance to plants against salinity stress. This technique not only improves the crop productivity under stress condition but also is an environmental friendly approach which is need of the time.

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