Pak. J. Bot., 46(1): 375-382, 2014.

INTEGRATED USE OF PLANT GROWTH PROMOTING RHIZOBACTERIA, BIOGAS SLURRY AND CHEMICAL NITROGEN FOR SUSTAINABLE PRODUCTION OF MAIZE UNDER SALT-AFFECTED CONDITIONS

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Abstract

Salinity is one of the most critical constraints hampering agricultural production throughout the world, including Pakistan. Some plant growth promoting rhizobacteria (PGPR) have the ability to reduce the deleterious effect of salinity on plants due to the presence of ACC-deaminase enzyme along with some other mechanisms. The integrated use of organic, chemical and biofertilizers can reduce dependence on expensive chemical inputs. To sustain high crop yields without deterioration of soil fertility, it is important to work out optimal combination of chemical and biofertilizers, and manures in the cropping system. A pot trial was conducted to study the effect of integrated use of PGPR, chemical nitrogen, and biogas slurry for sustainable production of maize under salt-stressed conditions and for good soil health. Results showed that sole application of PGPR, chemical nitrogen and biogas slurry enhanced maize growth but their combined application was more effective. Maximum improvement in maize growth, yield, ionic concentration in leaves and nutrient concentration in grains was observed in the treatment where PGPR and biogas slurry was used in the presence of 100% recommended nitrogen as chemical fertilizer. It also improved the soil pH, EC_e, and available N, P and K contents. It is concluded that integrated use of PGPR, biogas slurry and chemical nitrogen not only enhanced maize growth, yield and quality but also improved soil health. So, it may be evaluated under field conditions to get sustained yield of maize from salt-affected soils.

Introduction

Agriculture is the major sector for economic development of third world countries. Chemical fertilizers have become indispensable for agriculture but, presently, these are much expensive and, in certain cases, not available in time. Under salt-affected conditions, the crops require 25% more nitrogenous fertilizers than the normal soils (Mehdi *et al*., 2007). The integrated use of organic and chemical fertilizers can reduce dependence on expensive chemical inputs. To sustain high crop yields without deterioration of soil fertility, it is important to work out optimal combination of fertilizers and manures in the cropping system (Rekhi *et al*., 2000). The use of organic amendments is generally seen as a key issue for soil health and sustainability in cropping system (Timsina & Conner, 2001).

Cereals have major role in feeding world's population. Maize is the third major cereal crop of Pakistan and plays important role in value added in agriculture. Salinity is one of the most critical constraints hampering agricultural productions in many areas around the world including Pakistan. About one third of the irrigated land of Pakistan is prone to salinity and this figure is sharply increasing each year (Ashraf *et al*., 2008). Utilization of salt-affected soils for agriculture production is vital to feed the burgeoning population of Pakistan. In saline environment, plant growth is affected by complex interaction of hormones, osmotic effects, specific ion effect and nutritional imbalances, probably all occur simultaneously. According to biphasic model of crop growth response to salinity stress (Munns, 1993), salinity limits plant growth due to osmotic effect at first stage, while at second stage growth is affected due to accumulation of toxic ions (ion toxicity). This model may be used as a basis for inducing salt tolerance in maize (Schubert *et al*., 2009). As maize crop is more susceptible to salinity at vegetative stage than at later stages (Cramer, 1994) so inducing salinity tolerance in maize at first stage may be helpful to improve the physiological processes and thus improving crop yield. Plants under salinity stress produce elevated levels of ethylene which can damage the plants (Zapata *et al*., 2004) and results in poor plant growth due to the inhibition of root elongation. It is well documented that increasing Na^+ contents in soil cause an increase in Na^+ uptake and in general, decrease in K^+ contents of plant (Ashraf & Khanum, 1997; Ashraf, 2004). Therefore, massive uptake of a particular nutrient results in nutrient imbalance and deficiencies of certain other nutrients. Salt tolerance in plants depends mainly on the capability of root to decrease endogenous ethylene level and restrict or control uptake of Na^+. Therefore, a check on the accelerated ethylene concentration and suppression of Na^+ uptake by plant could be helpful in minimizing the negative effect of stress on plant.

Inoculation of crop plants with plant growth-promoting rhizobacteria (PGPR) is a contemporary agricultural practice used to improve crop yield (Nadeem *et al*., 2009, Ahmad *et al*., 2011). Plant growth-promoting rhizobacteria are soil-borne bacteria that can affect plant growth either directly through various mechanisms such as nitrogen fixation, solubilization of phosphorus and increasing growth by changing the endogenous level of plant growth regulating (PGR) substances (Shaharoona *et al*., 2006) or indirectly by increasing the natural resistance of the host against pathogens. Some PGPR strains have the ability to lower the level of ethylene in stressed plant and eliminate/reduce the potential inhibitory effect of high ethylene concentrations through development of better root system, thus help alleviating the stressed conditions in plants (Shaharoona *et al*., 2006, Nadeem *et al*., 2009, Ahmad *et al*., 2011). The use of plant growth promoting rhizobacteria increased the crop yield in addition to decrease in use of chemical fertilizers (Yasmin *et al.,* 2013).

The digested slurry contains organic nitrogen (mainly amino acids), abundant mineral elements, and low-molecular-mass bioactive substances like, hormones, humic acids and vitamins etc. (Liu *et al*., 2008). It may be used as organic manure and source of water as well. The fermented slurry which contains relatively high percentage of readily available nutrients can directly be applied in liquid and dried form to the plants both for basal and top dressing (Mikled *et al*., 1994). The bio slurry, beside its use as soil amendment for crop growth also prevents adverse environmental impacts of waste disposal (Garg *et al*., 2005). The increase in yield due to bio-slurry application has been reported for many crops. The crops produced with bio-slurry have better quality as compared to those produced with chemical fertilizer (Gurung, 1997).

In order to reduce dependence on commercial fertilizer, there is much interest to use local available farmyard manure as alternative source. Organic fertilizer cannot meet crop nutrient demand over large areas because of limited availability, low nutrient composition, and high labour requirement (Palm *et al*., 1998). The integrated use of different organic sources and chemical fertilizers increased the crop yield and improved the soil health by decreasing the salinity/ sodicity hazards under wheat-rice cropping system in recently reclaimed soil (Mehdi *et al.,* 2011). The integrated soil fertility management is the most feasible options to resource poor farmers in sustaining the productivity of maize (Mujeeb *et al.,* 2010). The combined use of organic wastes, biofertilizer and chemical fertilizers is beneficial in improving crop yield, soil pH, organic carbon and available N, P and K in sandy loam soil (Rautaray *et al.,* 2003; Soomro *et al.,* 2013). The effects of slurry and other organic manures in conjunction with chemical fertilizers on yields of a number of crops have been proved. These may be of potential benefits to farmers if they are suitably translated to the local situations, for both the manurial value of slurry and their potential application to different crops as dictated by numerous factors including animal and biogas plant feedstock, and application with different level of combinations (Gurung, 1997).

It is very likely that using biogas slurry enriched with PGPR in combination with inorganic fertilizer may be a sound soil fertility management strategy for improving agricultural productivity, particularly under salt-affected conditions. Apart from enhancing crop yields, the practice may have a greater beneficial residual effect that cannot be derived from the use of either organic or inorganic fertilizers applied alone. Additionally, these PGPR and organic fertilizers may be helpful in reclaiming salt-affected soils. Therefore, present experiment was conducted to study the effect of integrated use of PGPR and biogas slurry for improving the efficiency of chemical fertilizer in maize under salt-stressed conditions and to conserve the environment through improvement in soil health.

Materials and Methods

Collection of PGPR strains: Two PGPR strains carrying ACC-deaminase activity (S20; *Pseudomonas fluorescens* and S14; *Enterobacter aerogenes*), which have already shown their worth for inducing salt tolerance in maize (Nadeem *et al.,* 2009), were collected from Soil Microbiology and Biochemistry Lab., Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad.

Preparation of inocula: Inocula were prepared in Erlenmeyer flasks by using DF salt minimal medium containing ACC as substrate. Media was prepared by using the standard composition as described by Dworkin & Foster (1958). Each flask containing broth was inoculated with respective strains of PGPR and incubated at 28±1°C for 48 hours in shaking incubator (100 rpm). Optical density was measured at 540nm and uniform population (OD_{540} = 0.45; 10^7-10^8 cfu mL^{-1}) was achieved by dilution with sterilized water prior to seed inoculation.

Pot trial: A pot trial was conducted by using PGPR strains (S20; *Pseudomonas fluorescens* and S14; *Enterobacter aerogenes*), biogas slurry and chemical nitrogen fertilizer separately and in combinations. The biogas slurry (BGS) samples were collected from the biogas plants, air-dried and analyzed for NPK through standard procedures as described by Ryan *et al.,* (2001). The concentration of nitrogen, phosphorus and potassium in the BGS sample was 0.78, 1.01 and 0.91%, respectively. The experiment was conducted with following set of treatments: T1-control; T2-50% of recommended N; T3-100% of recommended N; T4-biofertilizer (P1 + P2), T5-biogas slurry @ 600 kg/ha; T6-(50% of recommended N + biofertilizer); T7-(100% of recommended N + Biofertilizer); T8-(50% of recommended N + BGS @ 600 kg/ha); T9-(100% of recommended N + BGS @ 600 kg/ha); T10-(50% of recommended N + BGS @ 600 kg/ha + biofertilizer); T11-(100% of recommended N + BGS @ 600 kg/ha + biofertilizer).

The soil used in pots was collected from salt-affected field, and analyzed for physico-chemical characteristics (Table 1). The soil was sandy loam in texture with EC_e and pH 7.1 dS m^{-1} and 8.43, respectively. Maize seeds were inoculated by dipping the seeds in broth culture for 10 minutes. Before inoculation, the broth cultures of both rhizobacterial strains were mixed in 1:1 ratio. In control, seeds were however, sown without dipping. The sieved soil was mixed with biogas slurry and chemical fertilizers prior to pot filling @ 12 kg per pot. Five maize seeds were sown in each pot and maintained one plant by thinning after15 days of germination. Pots were placed under ambient light and temperature conditions and standard irrigation quality criteria were followed to raise the crop (Ayers & Westcot, 1985). Recommended dose of P (80 kg ha^{-1}) and K (60 kg ha^{-1}), as triple super phosphate (TSP) and sulphate of potash (SOP), respectively, were applied in each pot along with N as urea according to treatment plan. All P and K fertilizers were applied as basal dose at the time of sowing, while nitrogen was applied in two equal splits viz. at sowing and 35 days after sowing. The data regarding growth and yield parameters were collected at physiological maturity. The post-harvest soil samples were collected and analyzed for pH, EC_e, and macronutrients (Ryan *et al.,* 2001).

Plant analyses: Leaf samples were collected after 60 days, digested according to the method of Wolf (1982) and analyzed for sodium (Na^+) and potassium (K^+) concentration through flame photometer as described by Ryan *et al*., (2001). Free proline contents were determined according to the method as described by Bates *et al*., (1973). Relative water contents (RWC) were measured by the method as described by Barrs & Weatherley (1962). Grain samples were digested (Wolf, 1982) and nitrogen was determined by Kjeldahl method (Ryan *et al*., 2001). The P contents were determined by spectrophotometer as described by Chapman & Prat (1961) and potassium concentration was measured through flame photometer (Ryan *et al*., 2001).

Statistical analysis: Analysis of variance techniques (ANOVA) were applied to analyze the data (Steel *et al*., 1997) using completely randomized design (CRD) and means were compared by Duncan's multiple range (DMR) test (Duncan, 1955).

Table 1. Physico-chemical characteristics of the soil used for pot trial.

Characteristic	Unit	Value
Sand	%	60.5
Silt	%	24.5
Clay	%	15.0
Textural class	-	Sandy loam
Saturation percentage	-	32.3
pH_s	-	8.43
EC_e	dS m^{-1}	7.12
Available phosphorus (Olson)	mg kg^{-1}	6.89
Extractable potassium (NH_4OAC)	mg kg^{-1}	64.0
Organic matter	%	0.47
Total nitrogen	mg kg^{-1}	26.38

Table 2. Effect of integrated use of Biofertilizer and biogas slurry on growth parameters of maize under salt-affected conditions.

Treatment	Plant height (cm)	Shoot weight (g)	Root length (cm)	Root weight (g)
T1	111.93 e	123.93 f	20.80 c	17.07 e
T2	126.83 de	144.97 e	27.00 b	19.43 de
T3	135.33 cd	159.30 c-e	27.90 ab	21.90 b-d
T4	141.83 b-d	162.83 c	30.67 ab	24.03 a-c
T5	133.43 cd	147.53 de	29.97 ab	18.03 e
T6	142.43 b-d	165.00 c	30.37 ab	23.33 bc
T7	169.17 a	186.10 b	31.13 ab	24.67 ab
T8	145.17 bc	160.33 cd	29.67 ab	21.37 cd
T9	156.00 ab	166.20 c	30.57 ab	23.50 a-c
T10	163.80 a	198.67 ab	31.80 ab	24.23 a-c
T11	171.33 a	212.80 a	33.50 a	26.47 a
LSD value ($p \leq 0.05$)	**15.742**	**14.769**	**5.9261**	**2.9823**

Means sharing same letters are statistically at par at 5 % level of probability. (n = 3)

T1-Control; T2-50% of Recommended N; T3-100% of Recommended N; T4-Biofertilizer (S14 + S20), T5-Biogas slurry @ 600 kg/ha; T6-(50% of Recommended N + Biofertilizer); T7-(100% of Recommended N + Biofertilizer); T8-(50% of Recommended N + BGS @ 600 kg/ha); T9-(100% of Recommended N + BGS @ 600 kg/ha); T10-(50% of Recommended N + BGS @ 600 kg/ha + Biofertilizer); T11-(100% of Recommended N + BGS @ 600 kg/ha + Biofertilizer)

Results

The results (Table 2) showed that sole application of PGPR and biogas slurry improved plant height at all nitrogen levels (0, 50 and 100% recommended N). The combined use of PGPR and biogas slurry was more effective for improving plant height at both nitrogen levels (50 and 100%) under salt-affected conditions. The maximum plant height (171.33 cm) was observed in T11, where PGPR (Biofertilizer) was used in combination with biogas slurry in the presence of 100% recommended nitrogen and the improvement was 53, 35 and 27% as compared to 0, 50 and 100% of recommended N, respectively. The integrated use of PGPR, chemical nitrogen, and biogas slurry also improved the shoot weight under salt-affected conditions significantly as compared to the treatments, where N was applied alone (Table 2). Maximum improvement in shoot weight was observed in T11, where PGPR (Biofertilizer) was used in combination with biogas slurry and 100% of recommended nitrogen. It was at par with treatment where biofertilizer + biogas slurry + 50% recommended N was applied.

Data regarding root length (Table 2) showed that separate application of PGPR and biogas slurry significantly improved the root length of maize grown under salt-affected conditions over the treatment T1, where no nitrogen was applied. However, the increase in root length due to PGPR and BGS was non-significant with treatments where 100% of recommended N was applied. The integrated use of PGPR, biogas slurry and 100% of recommended nitrogen (T11) was at par with all other treatments where PGPR and biogas slurry was used alone or in combination with chemical nitrogen. Root dry weight was also significantly improved by the sole application of PGPR and biogas slurry at all nitrogen levels (Table 2). The combined application, however, was more effective in improving the root dry weight of maize grown under salt-affected conditions, in the presence of 50 and 100% of the recommended level of chemical nitrogen. Maximum root dry weight (26.47 g) was again achieved by the combined use of PGPR, biogas slurry and 100% of recommended chemical nitrogen.

Data given in Table 3 showed that the combined use of PGPR, and biogas slurry in the presence of 100% recommended nitrogen was better in improving length and weight of maize cob, grown under salt-affected conditions as compared to the sole application of these. Maximum cob length (19.44 cm) and cob weight (164.13 g) was observed in T11, which were 43 and 17% higher than T3-(100% of recommended N). The application of PGPR and biogas slurry was also effective for improving the cob length and cob weight at 0, 50 and 100% of the recommended nitrogen levels.

Data regarding 100-grain weight (Table 3) showed that sole application of PGPR, chemical nitrogen, and biogas slurry resulted in more solid seeds as compared to control. The PGPR and biogas slurry again significantly improved 100-grain weight over T3, where 100% of recommended N was used. The combined use of PGPR and biogas slurry was more effective for improving grain weight at both nitrogen levels (50 and 100%) under salt-affected conditions. The maximum 100-grain weight (24.73 g) was observed in T11, where PGPR (Biofertilizer) was used in combination with biogas slurry and 100% of recommended nitrogen. It was non-significant with treatment T10 where PGPR and biogas slurry were applied with 50% of recommended N.

The integrated use of PGPR, BGS and chemical nitrogen (T11) significantly improved the grain yield plant^{-1} under salt-affected conditions as compared to control and the treatments where chemical nitrogen was applied alone (Table 3). In this treatment, maximum improvement in grain yield plant^{-1} was observed and it was at par with T6, T7, T9 and T10. The inoculation with biofertilizer in the absence of biogas slurry at 100% recommended nitrogen (T7) also significantly improved the grain yield plant^{-1} and the increase was 15% over T3 (un-inoculated control at 100% of recommended N).

Table 3. Effect of integrated use of Biofertilizer and biogas slurry on yield parameters of maize under salt-affected conditions.

Treatment	Cob length (cm)	Cob weight (g)	100-Grain weight (g)	Grain yield plant^{-1} (g)
T1	8.72 e	123.77 g	18.20 g	76.20 f
T2	12.01 d	134.13 ef	19.47 f	88.50 ef
T3	13.56 cd	140.67 de	20.53 ef	93.67 c-e
T4	14.52 c	133.63 ef	22.40 bc	94.13 c-e
T5	13.67 cd	131.67 f	19.70 f	89.27 de
T6	16.75 b	148.73 bc	22.17 cd	101.67 a-d
T7	17.12 b	154.00 b	23.53 ab	107.90 ab
T8	14.48 c	135.33 ef	21.07 de	98.70 b-e
T9	16.93 b	147.60 b-d	23.23 bc	102.47 a-c
T10	17.19 b	143.80 cd	23.63 ab	102.40 a-c
T11	19.44 a	164.13 a	24.73 a	112.00 a
LSD value ($p \leq 0.05$)	**2.1617**	**7.0393**	**1.2419**	**12.562**

Means sharing same letters are statistically at par at 5 % level of probability. (n = 3)
T1-Control; T2-50% of Recommended N; T3-100% of Recommended N; T4-Biofertilizer (S14 + S20), T5-Biogas slurry @ 600 kg/ha; T6-(50% of Recommended N + Biofertilizer); T7-(100% of Recommended N + Biofertilizer); T8-(50% of Recommended N + BGS @ 600 kg/ha); T9-(100% of Recommended N + BGS @ 600 kg/ha); T10-(50% of Recommended N + BGS @ 600 kg/ha + Biofertilizer); T11-(100% of Recommended N + BGS @ 600 kg/ha + Biofertilizer)

Data (Table 4) showed that PGPR gave significantly better results than biogas slurry for improving the relative water contents in maize plant at all nitrogen levels. The combined use of PGPR and biogas slurry in the presence of 100% of recommended nitrogen showed even better results for improving relative water contents. The highest relative water contents (74.87%) were observed in inoculated treatment with maximum N (T11) and it was significantly better than un-inoculated treatments at all levels of nitrogen.

The integrated use of PGPR, chemical nitrogen, and biogas slurry also significantly decreased the proline content in leaves of maize grown under salt-affected conditions (Table 4). Minimum proline contents (1.91 µmol g^{-1}) were produced with the combined use of PGPR and biogas slurry in the presence of 100% recommended chemical nitrogen.

The PGPR significantly improved the K^+/Na^+ ratio in leaves of maize grown under salt-affected conditions in the presence and absence of biogas slurry at all nitrogen levels (Table 4). Maximum K^+/Na^+ ratio (1.87) was observed in treatments, where PGPR and biogas slurry were used in the presence of 100% of the recommended chemical nitrogen (T11).

The results regarding the effect of PGPR, chemical nitrogen and biogas slurry on nutritional quality of maize grown under salt-affected conditions (Table 5) showed that application of fertilizers significantly improved the nitrogen, phosphorus and potassium contents in grains of maize over the control (T1). The combined use of PGPR, and biogas slurry in the presence of 100% recommended nitrogen showed significantly better results for improving the nutritional quality of maize grains and the increase was 25, 96 and 19%, respectively, over treatment where 100% of recommended N was applied (T3).

Table 4. Effect of integrated use of Biofertilizer and biogas slurry on relative water content, proline content, and K^+/Na^+ ratio in leaves of maize under salt-affected conditions.

Treatment	Relative water content (%)	Proline content (µmol g^{-1})	K^+/Na^+ ratio in leaves -
T1	60.17 e	2.30 a	1.45 d
T2	62.20 e	2.27 ab	1.50 cd
T3	66.13 d	2.25 a-c	1.54 cd
T4	72.17 ab	2.13 d	1.73 b
T5	67.13 d	2.29 a	1.58 c
T6	73.23 ab	2.10 d	1.77 ab
T7	73.83 a	2.02 e	1.82 ab
T8	68.27 cd	2.22 bc	1.60 c
T9	70.87 bc	2.20 c	1.59 c
T10	72.60 ab	1.97 ef	1.73 b
T11	74.87 a	1.91 f	1.87 a
LSD value ($p \leq 0.05$)	**2.7631**	**0.0568**	**0.1057**

Means sharing same letters are statistically at par at 5 % level of probability. (n = 3)
T1-Control; T2-50% of Recommended N; T3-100% of Recommended N; T4-Biofertilizer (S14 + S20), T5-Biogas slurry @ 600 kg/ha; T6-(50% of Recommended N + Biofertilizer); T7-(100% of Recommended N + Biofertilizer); T8-(50% of Recommended N + BGS @ 600 kg/ha); T9-(100% of Recommended N + BGS @ 600 kg/ha); T10-(50% of Recommended N + BGS @ 600 kg/ha + Biofertilizer); T11-(100% of Recommended N + BGS @ 600 kg/ha + Biofertilizer)

Table 5. Effect of integrated use of Biofertilizer and biogas slurry on nitrogen, phosphorus, and potassium contents in grains of maize under salt-affected conditions.

Treatment	Nitrogen in grains	Phosphorus in grains	Potassium in grains
		(%)	
T1	1.24 f	0.22 e	1.41 d
T2	1.31 e	0.24 de	1.42 d
T3	1.38 d	0.26 de	1.47 d
T4	1.43 d	0.36 c	1.54 c
T5	1.39 d	0.28 d	1.45 d
T6	1.53 c	0.35 c	1.66 b
T7	1.62 b	0.45 b	1.72 ab
T8	1.44 d	0.27 d	1.47 d
T9	1.50 c	0.33 c	1.58 c
T10	1.64 b	0.42 b	1.69 ab
T11	1.72 a	0.51 a	1.75 a
LSD value ($p \leq 0.05$)	**0.0631**	**0.0472**	**0.0644**

Means sharing same letters are statistically at par at 5 % level of probability. (n = 3)
T1-Control; T2-50% of Recommended N; T3-100% of Recommended N; T4-Biofertilizer (S14 + S20), T5-Biogas slurry @ 600 kg/ha; T6-(50% of Recommended N + Biofertilizer); T7-(100% of Recommended N + Biofertilizer); T8-(50% of Recommended N + BGS @ 600 kg/ha); T9-(100% of Recommended N + BGS @ 600 kg/ha); T10-(50% of Recommended N + BGS @ 600 kg/ha + Biofertilizer); T11-(100% of Recommended N + BGS @ 600 kg/ha + Biofertilizer)

The results of post-harvest soil analyses (Table 6) showed that crop growth decreased the salinity level of the soil and improved its nutritional status. The separate application of PGPR and biogas slurry decreased the pH of the maize rhizosphere irrespective of applied nitrogen under salt-affected conditions, but this decrease was non-significant when compared with control. The integrated use of PGPR and biogas slurry at 50 and 100% nitrogen levels significantly decreased soil pH when compared with un-inoculated treatments and the decrease was non-significant to that with 100% of recommended chemical nitrogen application. The separate as well as combined use of PGPR and biogas slurry significantly decreased EC_e of the soil (Table 6). The maximum decrease in soil EC_e was observed in treatments where PGPR, BGS and chemical N were integrated (T11). The soil macronutrients concentration was also improved with the application of PGPR and biogas slurry in the presence and absence of nitrogen under salt-affected conditions. Maximum nitrogen, phosphorus and potassium concentration was observed in treatment where PGPR and biogas slurry were used in the presence of 100% of the recommended chemical nitrogen, and the increase was 63, 60 and 21%, respectively, over the treatment where 100% of recommended N was applied.

Table 6. Effect of integrated use of Biofertilizer and biogas slurry on post-harvest soil pH, EC_e (dS m^{-1}) and macronutrients (mg kg^{-1}).

Treatment	pH	EC_e	Nitrogen	Phosphorus	Potassium
		dS m^{-1}	mg kg^{-1}		
T1	8.24 a	6.65 a	14.83 i	9.89 g	71.28 f
T2	8.23 a	6.29 b	23.46 gh	15.98 f	79.14 e
T3	8.20 a-c	5.95 c	37.62 de	28.82 e	86.78 c-e
T4	8.21 ab	5.72 d	20.15 h	33.53 d	83.46 e
T5	8.21 ab	5.80 cd	26.95 fg	31.69 de	80.61 e
T6	8.18 a-c	5.54 ef	29.94 f	38.63 b	92.38 b-d
T7	8.16 a-c	5.44 fg	41.17 cd	41.32 b	98.55 ab
T8	8.19 a-c	5.66 de	35.75 e	34.16 cd	84.73 de
T9	8.17 a-c	5.46 fg	47.25 b	38.41 bc	93.62 bc
T10	8.15 bc	5.28 gh	43.81 bc	40.81 b	101.96 a
T11	8.12 c	5.22 h	61.22 a	46.07 a	104.95 a
LSD value ($p \leq 0.05$)	**0.0803**	**0.1795**	**5.2213**	**4.4318**	**7.7583**

Means sharing same letters are statistically at par at 5 % level of probability. (n = 3)

T1-Control; T2-50% of Recommended N; T3-100% of Recommended N; T4-Biofertilizer (S14 + S20), T5-Biogas slurry @ 600 kg/ha; T6-(50% of Recommended N + Biofertilizer); T7-(100% of Recommended N + Biofertilizer); T8-(50% of Recommended N + BGS @ 600 kg/ha); T9-(100% of Recommended N + BGS @ 600 kg/ha); T10-(50% of Recommended N + BGS @ 600 kg/ha + Biofertilizer); T11-(100% of Recommended N + BGS @ 600 kg/ha + Biofertilizer)

Discussion

Biogas slurry (BGS) contains a considerable amount of nutrients besides appreciable quantities of organic matter. Due to changing scenario of soil fertility management, the biogas slurry plays a vital role in restoring soil fertility. Biogas slurry is environmental friendly, has no toxic or harmful effects and ensures minimal dependence on chemical fertilizers. Additionally, nutrients from organic sources are more efficient than those from chemical sources. Moreover, under salt-affected conditions, the nitrogen fertilizer requirement of crops increases up to 125% (Mehdi *et al*., 2007) as fertilizer use efficiency decreases under these circumstances. The reduction in deleterious effects of stress-induced ethylene by plant growth promoting rhizobacteria containing ACC-deaminase has been well documented (Shaharoona *et al*., 2006; Nadeem *et al*., 2009; Ahmad *et al*., 2011). Organic fertilizer cannot meet crop nutrient demand over large areas because of limited availability, low nutrient composition, and high labour requirement (Palm *et al*., 1998; Ahmad *et al*., 2012) therefore; integrated use of available nutrient resources is the most feasible option to resource poor farmers for sustainable maize production. A pot experiment was conducted to evaluate the effect of integrated use of PGPR, chemical nitrogen, and biogas slurry for sustainable production of maize under salt-stressed conditions and to improve soil health.

Results of our study showed that sole use of PGPR containing ACC-deaminase and biogas slurry enhanced maize growth and yield under salt-affected conditions but their combined application was more effective. This may be attributed to the enhanced nutrient uptake due to the proliferated roots through growth promoting activity of PGPR through ACC-deaminase activity along with some other mechanisms (Nadeem *et al*., 2009; Ahmad *et al*., 2011, 2012; El Husseini *et al*., 2012). This may also be due to improvement in soil chemical properties and nutritional status, and increased microbial activity in the root zone due to the integrated effects of biogas slurry and PGPR. The increase in crop growth and yield due to application of biogas slurry has been reported in previous studies (Islam *et al*., 2009; Baldi *et al*., 2010). Similarly, the integrated use of organic, inorganic and biofertilizers improved the crop growth and yield under normal conditions (Khaliq *et al*., 2006; Jilani *et al*., 2007; Koushal & Singh, 2011). The digested slurry contains organic nitrogen, readily available nutrients, hormones, humic acids and vitamins etc. (Mikled *et al*., 1994; Liu *et al*., 2008) which can enhance plant growth and yield through enhanced nutrient availability and improved soil health. The increase in growth and yield due to combined use of organic matter and biofertilizer has been reported by Iqbal *et al*. (2013) for lentil crop.

The integrated use of biogas slurry, PGPR and chemical nitrogen also decreased the proline content, and improved relative water content along with K^+/Na^+ ratio in plant tissues. This might be due to the decrease in deleterious effects of salinity by the PGPR through ACC-deaminase activity and restricted uptake of Na^+ resulting into enhanced K^+ uptake. It was in line with many previous studies (Nadeem *et al*., 2009; Ahmad *et al*., 2012). The biogas slurry might have improved the soil health thus, more nutrient availability, more microbial activity that results into more root proliferation and water uptake thus enhanced plant growth and yield.

The integrated use of available nutrient resources also improved the quality of maize grown under salt-affected conditions. This may be due to the plant growth promoting characteristics of PGPR in addition to the beneficial effects of biogas slurry on soil nutrient status, water holding capacity and other physico-chemical properties of the soil. The improvement in quality attributes of crops due to integrated use of organic and biofertilizers has also been reported in the previous studies (Jilani *et al*., 2007; Kumar *et al*., 2011; Iqbal *et al*., 2013). The better quality crops can be produced with the application of biogas slurry (Gurung, 1997).

Under salt-affected soils, the combined use of PGPR and biogas slurry decreased soil pH and EC_e, and improved nutrient status. This might be due to the production of organic acids by PGPR and more microbial activity. It might also be attributed to the solubilization of phosphorus by these PGPR (Nadeem *et al*., 2009; Ahmad *et al*., 2011) and increased surface area of roots through enhanced root proliferation (El-Husseini *et al*., 2012). It has been documented that combined use of organic and chemical fertilizers is beneficial in improving soil pH and available nutrients status (Rautaray *et al*., 2003).

Conclusion

It is concluded that integrated use of PGPR, biogas slurry and chemical nitrogen is a useful strategy to enhance growth, yield and quality of maize crop under salt-affected conditions through improvement of soil health and nutritional status. For more comprehensive results, the concept should be evaluated under salt-affected field conditions to develop valued recommendations.

Acknowledgement

We are highly thankful to Higher Education Commission, Pakistan and the Islamia University of Bahawalpur for providing the financial support to execute this research project.

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(Received for publication 18 September 2012)