*Soil Environ. 36(1): 59-69, 2017*
*DOI: 10.25252/SE/17/31043*
*www.se.org.pk*
*Online ISSN: 2075-1141*
*Print ISSN: 2074-9546*

# Evaluation of biofertilizer in combination with organic amendments and rock phosphate enriched compost for improving productivity of chickpea and maize

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## Abstract

*Chickpea has the potential to increase nitrogen nutrition of subsequent cereal crop in chickpea-maize cropping system. As a legume, chickpea takes much of its required nitrogen from atmosphere through symbiotic association with Rhizobium (soil bacteria), and thus least dependent on chemical N fertilizer. After harvesting, N captured in chickpea roots stays in soil for following cereal crop. Maize and chickpea are major crops of Pakistan and important dietary products people. Biofertilizers are products of living microorganisms with potential to improve productivity of crops through a number of direct and indirect mechanisms in environment friendly and sustainable manner. Therefore, a biofertilizer with specific bacterial strains for chickpea and maize, rock phosphate (RP) enriched compost and biogas slurry was acquired and analyzed for various physicochemical properties in laboratory. A pot experiment was conducted to evaluate the biofertilizer, RP-compost and biogas slurry for improving growth and yield of chickpea. Afterwards, maize was planted in the same pots containing chickpea roots. Same treatment combinations were applied to both crops. Growth, physiology, yield and chemical parameters were recorded for chickpea and maize crops. Application of biofertilizer gave significantly better results and improved grain yield in chickpea (40%) and in maize (14%) crop as compared to control. The use of biofertilizer in combination with biogas slurry can be suggested as economically effective and sustainable approach for improving soil health and productivity of chickpea and maize crops.*

**Keywords:** biofertilizer, biogas slurry, productivity, sustainability

## Introduction

Maize and chickpea cropping system is beneficial in terms of soil health and agricultural production. Legumes play a vital role in the cropping system of developing countries of arid and semi-arid regions for improving soil fertility and increasing crop productivity. Chickpea can successfully be grown as alternative food crop under water limited conditions due to less water requirements. Chickpea occupies 20% of the world pulse production (Aslam, 2004).

Cereals have major role in feeding the world's population. Cereals fulfill about 50% of nutritional requirement of people. Maize is the third major cereal crop of Pakistan and is the major staple food for most of the world population. Maize is an exhaustive crop so it desires more nutrient and water as compared to legumes. Whereas legumes improve soil fertility and physical conditions for better plant growth. However, low yield is common problem in farming systems which is due to deterioration of soil fertility and reduced N fixation by legumes.

In cropping system where affordability and accessibility of fertilizer is a problem, legumes may play vital role. Chemical fertilizers are economically important for cereal production and necessary to reach the nutritional demands for human consumption. Including legumes in cropping system can enhance symbiotic nitrogen fixation and improve phosphorus level of soil as well (Sinclair and Vadez, 2012). Growing of legumes has significant effect on the yield of subsequent maize (McDonagh *et al.,* 1993; Phoomthaisong *et al.,* 2003). Chickpea can improve soil fertility through biological nitrogen fixation from the atmosphere in association with symbiotic bacteria (Herridge *et al.,* 2008).

Biological nitrogen fixation plays a vital role for sustainable legumes production and important source of N for farmers. Legume seed inoculated with *Rhizobium* shows better growth, nitrogen fixation, seed yield and also improve nutrient uptake (Mfilinge *et al*., 2014). Similarly, minimizing soil disturbance in different cropping systems is essential for sustainable grain production (Franchini *et al*., 2012). A legume helps to maintain soil fertility and organic matter.

Chemical fertilizers have become indispensable for agriculture but, presently, these are much expensive and, in certain cases, not available in time. Biofertilizers are the

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substances that contain living microbes, when applied to soil either by soil application or seed priming, colonize the rhizosphere. These applied microbes interact with plants and improve growth and yield with their different growth promoting mechanisms.

Microorganisms and plant's secreted low molecular weight complex substances (siderophores) in the rhizosphere help plants for acquiring iron and other micronutrients uptake from rhizosphere (Bashan and Lavanony, 1990). Microbes used as biofertilizer are isolated from soil and characterized under laboratory conditions, and are applied to crops by different methods (Cakmakçi *et al.,* 2006). When soluble phosphate is applied to soil, soon after its application, it becomes fixed in insoluble form (Rodriquez and Fraga, 1999). Bacteria such as *Bacillus* and *Pseudomonas* have the ability to convert this insoluble phosphate into soluble form by secreting organic acids (Vazquez *et al*., 2000: Khan *et al.,* 2009). These kind of microbial inoculants are used as biofertilizers that can enhance plant growth and productivity as well as nutrient status of host plant.

Increased root growth also results in more infection sites available for Rhizobium. Biofertilizers having ACC-deaminase containing PGPR and rhizobia develop a strong interaction during the process of root colonization, improve nodulation and $N_2$ fixation in leguminous plants (Barea *et al.,* 2005). This enhanced biological nitrogen fixation increases protein content in legume grains, thus improves the quality of grains. Plants that are inoculated with PGPR containing ACC-deaminase are more resistant to deleterious effects of stressful conditions (Nadeem *at al.,* 2009; Ahmad *et al.,* 2011). Therefore, inoculation of seed or roots with specific inoculants could suppress endogenous ethylene synthesis, which subsequently creates physiological response and increase tolerance of crops to stress through lowering endogenous ethylene production and consequently improving nodulation in legumes (Ahmad *et al.,* 2012; 2013).

Application of organic manure also improves the growth and yield of crops. The use of farm yard manure (FYM) in cereal- legume cropping system has been well documented to improve yield and yield components of maize (Arif *et al*., 2011). Microbes when used with chemical fertilizers significantly increase growth and yield of crops (Shamma and Shahwany, 2014). The integrated use of plant growth promoting bacteria (PGPR) with p-enriched compost was found highly effective in improving growth, yield and nodulation of chickpea (Shahzad *et al*., 2008). So, the use of PGPR is helpful to enhance plant growth and crop yield.

Keeping in view the above discussion, there is a need to explore and check out maximum potential of legumes and their residual effect on soil fertility and crop productivity with integrated use of biofertilizer and organic sources in the region to find out their feasibility to be used by farmers for better productivity and profitability. The present experiments were therefore, conducted to evaluate the agronomic effectiveness of biofertilizer in combination with organic sources for improving the productivity and quality of chickpea and maize crops in pot experiment.

## Materials and Methods

Two pot experiments were conducted for evaluation of already prepared biofertilizer in combination with organic amendments (RP-enriched compost and biogas slurry) for improving the productivity of chickpea and maize crops.

## Collection and analysis of biofertilizer

Biofertilizer (Rhizogold) specific for chickpea and maize crops was obtained from Soil Microbiology and Biochemistry Laboratory, Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad-Pakistan. Before applying to crops, bacterial population of biofertilizer was tested in laboratory. Colony forming units (CFU) of bacteria was enumerated by standard serial dilution method as described by Alexander (1982). The population of bacteria was also confirmed by MPN method (Alexander and Clark, 1985) and that was $10^7$-$10^8$ CFU/ mL.

## Collection and analysis of rock-phosphate enriched compost (RP-EC)

Rock-phosphate enriched compost was obtained from Environmental Sciences Laboratory, Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad-Pakistan. The chemical analysis of the RP-EC was done by using standard methods. Carbon contents of RP-EC were estimated by loss-on-ignition method (Nelson and Sommers, 1999; Ryan *et al.,* 2001). Total nitrogen (N) contents were determined by using Kjeldahl distillation apparatus (Jackson, 1962). The total P contents were determined by taking the absorbance using spectrophotometer (Beckman photometer 1211). The digested filtrate was used for the determination of K contents using Jenway PFP-7 flame photometer. The physiochemical characteristics are given in Table 1.

## Collection and analysis of biogas slurry

Biogas slurry was obtained through the outlet of biogas plant of cattle dung, which was already installed in Chak # 8BC, Bahawalpur. The physicochemical properties of biogas slurry (Table 1) were determined using the standard methods as described by Ryan *et al.* (2001).

**Table 1: The chemical analysis of the rock phosphate enriched compost (RP-EC) & biogas slurry**

| Characteristic | Unit | Rock phosphate enriched compost | Biogas slurry |
|---|---|---|---|
| pH | | 6.87 | 4.51 |
| Organic matter | % | 48 | 45.5 |
| Carbon | % | 28 | 26.5 |
| Nitrogen | % | 1.4 | 1.2 |
| Phosphorous | % | 3.1 | 1.4 |
| Potassium | % | 1.6 | 1.79 |

## Pot experiments

## Evaluation of biofertilizer in combination with organic amendments for improving productivity of chickpea and maize crops

The experiments were conducted in wire house of the Department of Soil Science, University College of Agriculture and Environmental Sciences, The Islamia University of Bahawalpur, Pakistan. The main objective of study was to evaluate the effect of biofertilizer in combination with organic sources for improving growth, physiology, yield and chemical parameters of chickpea and maize crops. First, pot experiment was conducted by using chickpea as test crop. Soil for pot the experiment was taken from surface layer of experimental fields of Department of Soil Science, University College of Agriculture and Environmental Sciences, The Islamia University of Bahawalpur, Pakistan. Soil was air dried, ground, and passed through a 2 mm sieve, and pots were filled with 12 kg soil. Chickpea variety Bittle 98 was used as test crop in the experiment. Soil used in pots was analyzed for physical and chemical characteristics (Table 2). Then, maize pot trial was conducted on the previously harvested chickpea crop soil in the wire house. Same treatments and same procedure was adopted for maize experiment but biofertilizer used for maize crop was specific. Five seeds of maize variety (Poineer 30Y87) were sown in each pot which was maintained to three plants per pot after thinning.

Experiments comprised of 6 treatments with six replications for chickpea while three replications for maize crop following Completely Randomized Design (CRD). The treatments were control, biogas slurry, RP-EC, biofertilizer (Rhizogold), biogas slurry + biofertilizer (Rhizogold), and RP-EC + biofertilizer (Rhizogold). Different treatments (biogas slurry, phosphorus enriched compost and biofertilizer (Rhizogold) alone and in combination) were applied before sowing.

**Table 2: Physicochemical characteristics of the soil used for pot trials of chickpea and maize crops**

| Characteristic | Unit | Value |
|---|---|---|
| Textural class | -- | Sandy loam |
| Saturation percentage | % | 42 |
| pH | -- | 7.8 |
| EC | dS $m^{-1}$ | 1.4 |
| Organic matter | % | 0.57 |
| Total nitrogen | % | 0.03 |
| Available phosphorous | mg $kg^{-1}$ | 3.4 |
| Available potassium | mg $kg^{-1}$ | 84 |

Biogas slurry was applied at 600 kg $ha^{-1}$, RP-EC was applied at 100 kg $ha^{-1}$, as it contains 14% P, so deducted 14% chemical fertilizer in the treatments where RP-EC was applied. Recommended dose of P and K @ 60 kg $ha^{-1}$ each while half of the recommended dose of nitrogen (20 kg $ha^{-1}$) was applied as basal dose for chickpea. For maize crop, recommended dose of NPK (175 kg $ha^{-1}$, 160 kg $ha^{-1}$ and 125 kg $ha^{-1}$) was applied. 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 in maize crop. For both crops, fertilizers were applied as Urea, DAP and SOP as sources of N, P and K, respectively. Soil in each pot was remixed to homogenize it with respect to NPK. Ten chickpea and five maize seeds were sown in each pot and maintained to three plants 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 and Westcot, 1985). Pots were irrigated with tap water as and when required to maintain optimum moisture for plant growth. Chickpea–maize cropping system was followed i.e. chickpea was sown in October followed by maize by the end of June.

## Plant growth measurements

At various stages of chickpea and maize crop, data regarding growth, physiological, yield and chemical parameters were recorded during growth period and after harvesting of crop.

## Data recording

Number of nodules per plant, nodule fresh weight and nodule dry weight were recorded in chickpea crop. Plant height was taken at maturity with measuring tape. Shoot and root fresh and dry biomass were recorded after harvesting of chickpea. All standard growth parameters were measured in maize crop. Grain yield per pot and 100 grain weight was recorded for chickpea and maize crops as well.

Data regarding physiological parameters in chickpea was measured following the method of Hiscox and Israelstam (1979) for chlorophyll “a” and Arnon (1949) for the chlorophyll “b”. However, in maize crop chlorophyll contents were measured (10:15 to 11:30 hrs) by using chlorophyll meter (SPAD-502, Konica Minolta, Japan). Relative water contents (RWC) of shoot of chickpea and maize crop were determined using the formula as described by Mayak *et al.* (2004a).

The oven dried grain samples of chickpea and maize separately were ground and 0.1 g of samples was taken in digestion tubes for digestion using sulfuric acid ($H_2SO_4$) and hydrogen peroxide ($H_2O_2$) according to the method of McGill and Figueiredo (1993). Nitrogen was determined from plant filtrate using Kjeldhal method (Jackson, 1962). The phosphorus was determined according to Ashraf *et al.* (1992). Potassium in grain sample was determined by flame photometer according to method as described by Chapman and Parker (1961).

For iron, grain samples (1 g each) were ground in a mill (IKA Werke, MF 10 Basic, Staufen, Germany) to pass through a 0.5-mm sieve. Grain samples were digested in a di-acid mixture ($HNO_3$:$HClO_4$ ratio of 2:1) for analysis (Jones *et al.*, 1990). Iron concentrations in the digest were measured by atomic absorption spectrophotometer (PerkinElmer, AAnalyst 100, Waltham, USA). The Zn was also determined by atomic absorption spectrometer (PerkinElmer, Aanalyst 100, Waltham, USA) from the digested samples. The post-harvest soil samples were collected and analyzed for N in soil and biological characteristics (Ryan *et al.*, 2001).

## Statistical analysis

Data regarding growth, physiological, yield and chemical parameters of chickpea and maize were analyzed statistically using software “Statistix 8.1®” for analysis of variance (ANOVA) and means were compared by using least significant difference (LSD) test at probability level P≤0.05 (Steel *et al.*, 1997).

## Results

## Nodulation and physiological parameters of chickpea

The data showed that sole as well as combined application of biofertilizer (rhizogold), biogas slurry and RP-EC showed significant increase in growth (number of nodules plant$^{-1}$, nodule fresh weight, nodule dry weight) and physiological (relative water content, chlorophyll a, chlorophyll b) parameters of chickpea as compared to control (Table 3). Application of biofertilizer along with biogas slurry improved number of nodules plant$^{-1}$, nodule fresh weight, nodule dry weight, relative water content, chlorophyll a and chlorophyll b up to 121, 144, 170, 16, 13 and 13%, respectively, as compared to control.

## Growth and yield parameters of chickpea

Sole application of biofertilizer, biogas slurry and phosphorus enriched compost significantly improved growth and yield parameters of chickpea. However, most prominent results were observed with the application of biofertilizer in combination with biogas slurry in overall growth and yield parameter as compared to control (Table 4). The biofertilizer in combination with biogas slurry improved plant height (26%), shoot dry biomass (72%), root fresh biomass (70%), root dry biomass (120%), 100 grain weight (6%) and yield per pot (40%) as compared to control.

## Grain quality and soil health after harvesting of chickpea

All the applied treatments (biofertilizer, biogas slurry, RP-EC) showed higher grain quality parameters (N, P and K concentration in grain) and soil parameters after harvesting of chickpea (N in soil and CFU) as compared to control (Table 5). However, biofertilizer in combination with biogas slurry performed better than all other treatments and it improved N in grain (4%), P in grain (41%), K in grain (6%), N in soil (9%) and CFU of bacteria in soil (49%) as compared to control.

## Growth parameters of maize

Individual as well as combined application of biofertilizer, biogas slurry and RP-EC significantly improved overall growth parameters (plant height, root length , shoots fresh biomass , shoots dry biomass , root fresh biomass, root dry biomass, number of leaves per plant) of maize crop as compared to control (Table 6). Application of biofertilizer in combination with biogas slurry showed significantly higher results than individual application of biofertilizer, biogas slurry and RP-enriched compost. The combined application of biofertilizer with biogas slurry improved plant height (6%), root length (32%), shoot fresh biomass (5%), shoot dry biomass (68%), root fresh biomass (73%), root dry biomass (78%), number of leaves per plant (13%) of maize plant in pot trial as compared to control.

### Physiological and yield parameters of maize

Maximum relative water content, chlorophyll content, 100 grain weight, grain yield per pot and Stover yield per cob of maize crop in pot trial was found with combined application of biofertilizer and biogas slurry that was 10, 65, 7, 14 and 46% more as compared to control (Table 7).

Overall, physiological and yield parameters were significantly improved by sole as well as combined application of biofertilizer, biogas slurry and RP-EC.

**Table 3: Effect of biofertilizer in combination with organic amendments on nodulation and physiological parameters of chickpea**

| Treatment | Number of nodules plant$^{-1}$ | Nodule fresh weight (g plant$^{-1}$) | Nodule dry weight (g plant$^{-1}$) | Relative water content (%) | Chlorophyll a (mg g$^{-1}$) | Chlorophyll b (mg g$^{-1}$) |
|---|---|---|---|---|---|---|
| T1: Control | 24.83 $^{c}$ | 0.72 $^{c}$ | 0.15 $^{c}$ | 60.67 $^{d}$ | 1.327 $^{c}$ | 0.677 $^{a}$ |
| T2: Biogas Slurry (BGS) | 36.07 $^{b}$ (45) | 1.13 $^{b}$ (57) | 0.23 $^{b}$ (49) | 63.67 $^{c}$ (5) | 1.393 $^{b}$ (5) | 0.720 $^{a}$ (6) |
| T3: Enriched Compost (EC) | 31.37 $^{bc}$ (26) | 1.04 $^{b}$ (45) | 0.21 $^{c}$ (34) | 62.33 $^{cd}$ (3) | 1.337 $^{b}$ (4) | 0.713 $^{a}$ (5) |
| T4: Biofertilizer | 48.37 $^{a}$ (95) | 1.58 $^{a}$ (118) | 0.30 $^{b}$ (95) | 66.33 $^{bc}$ (9) | 1.460 $^{a}$ (10) | 0.743 $^{a}$ (10) |
| T5: BGS + Biofertilizer | 55.00 $^{a}$ (121) | 1.74 $^{a}$ (144) | 0.41 $^{a}$ (170) | 70.33 $^{a}$ (16) | 1.497 $^{a}$ (13) | 0.767 $^{a}$ (13) |
| T6: EC + Biofertilizer | 54.77 $^{a}$ (121) | 1.77 $^{a}$ (141) | 0.41 $^{a}$ (165) | 69.33 $^{ab}$ (14) | 1.487 $^{a}$ (12) | 0.740 $^{a}$ (9) |
| LSD Value | 10.019 | 0.2829 | 0.0761 | 3.0813 | 0.0415 | 0.1068 |
| **CV** | 13.49 | 11.96 | 15.03 | 2.65 | 1.64 | 8.26 |

**Table 4: Effect of biofertilizer in combination with organic amendments on growth and yield parameters of chickpea**

| Treatment | Plant height (cm) | Shoots dry biomass (g pot$^{-1}$) | Root fresh biomass (g pot$^{-1}$) | Root dry biomass (g pot$^{-1}$) | 100 grain weight (g) | Grain yield (g pot$^{-1}$) |
|---|---|---|---|---|---|---|
| T1: Control | 46.69 $^{c}$ | 11.51 $^{d}$ | 3.87 $^{d}$ | 0.65 $^{c}$ | 24.8 $^{c}$ | 29 $^{d}$ |
| T2: Biogas Slurry (BGS) | 51.71 $^{bc}$ (11) | 16.65 $^{c}$ (45) | 5.40 $^{bc}$ (40) | 1.00 $^{b}$ (53) | 25.6 $^{abc}$ (3) | 33 $^{cd}$ (15) |
| T3: Enriched Compost (EC) | 50.26 $^{c}$ (8) | 15.32 $^{c}$ (33) | 5.16 $^{c}$ (33) | 1.03$^{b}$ (58) | 25.4 $^{bc}$ (2) | 32 $^{cd}$ (9) |
| T4: Biofertilizer | 56.76 $^{ab}$ (22) | 18.22 $^{b}$ (58) | 6.07 $^{ab}$ (57) | 1.27 $^{ab}$ (95) | 26.0 $^{ab}$ (5) | 35 $^{bc}$ (22) |
| T5: BGS + Biofertilizer | 58.68 $^{a}$ (26) | 19.75 $^{a}$ (72) | 6.57 $^{a}$ (70) | 1.43 $^{a}$ (120) | 26.4 $^{a}$ (6) | 41 $^{a}$ (40) |
| T6: EC + Biofertilizer | 57.52 $^{a}$ (23) | 18.92 $^{ab}$ (64) | 6.22 $^{ab}$ (61) | 1.36 $^{a}$ (109) | 26.3 $^{a}$ (6) | 40 $^{ab}$ (37) |
| LSD Value | 5.3623 | 1.4908 | 0.9087 | 0.2869 | 0.8365 | 4.3374 |
| CV | 5.62 | 5.01 | 9.21 | 14.33 | 1.83 | 6.98 |

( ) indicates % increase from the control; *Means followed by the same letters are not statistically different at $P<0.05$ according to the least significant difference (LSD) test

## Grain quality and soil health after harvesting of maize

Individual application of biofertilizer, biogas slurry and RP-EC significantly improved grain quality parameters (N, P, K, Fe and Zn concentration in grain) and soil parameters after harvesting of maize (N in soil and bacterial CFU) as compared to control (Table 8). However, combined application of biofertilizer and biogas slurry was more effective than individual application and improved N, P, K, Fe and Zinc in grain, and N and bacterial CFU in soil up to 29, 53, 18, 37, 31, 9 and 73% as compared to control.

## Discussion

The combined use of organic wastes, biofertilizer and chemical fertilizers is beneficial for improving crop yield, soil pH, organic carbon and available nutrients (Rautaray *et al.*, 2003; Ahmad *et al*., 2014). The effects of organic amendment in conjunction with chemical amendments on the yields of a number of crops and soil health have been proved better (Mantovi *et al.*, 2005). These may be of potential benefits to farmers if they are suitably translated to local situations and applied to different crops as dictated by numerous factors including animal and biogas plant waste, and application with different level of combinations (Gurung, 1997). The application of RP-EC alone or in combination with biofertilizer could be helpful in improving the nodulation, growth and yield of crop plants. There are few reports about the enrichment of compost with phosphorus. Shahzad *et al.* (2008) formulated phosphorus enriched compost with PGPB and found that combined application resulted in an increased growth and yield of crops compared to control. Recently, Saleem *et al.* (2013) found increase in growth and yield of wheat due to application of compost.

**Table 5: Effect of biofertilizer in combination with organic amendments on grain quality and soil health after harvesting of chickpea**

| Treatment | N concentration in grain (%) | P concentration in grains (%) | K concentration in grains (%) | Nconcentration in soil after harvesting (%) | Bacterial population after harvesting (cfu × $10^{-4}$) |
|---|---|---|---|---|---|
| T1: Control | 2.72 $^{d}$ | 0.48 $^{d}$ | 1.50 $^{b}$ | 0.025 $^{b}$ | 2.9 $^{b}$ |
| T2: Biogas Slurry (BGS) | 2.75 $^{bcd}$ (1) | 0.57 $^{bc}$ (19) | 1.53 $^{ab}$ (2) | 0.025 $^{b}$ (3) | 3.2 $^{b}$ (9) |
| T3: Enriched Compost (EC) | 2.74 cd (1) | 0.52 $^{cd}$ (9) | 1.54 $^{ab}$ (3) | 0.025 $^{b}$ (1) | 3.0 $^{b}$ (3) |
| T4: Biofertilizer | 2.79 abc (3) | 0.61 $^{ab}$ (27) | 1.56 $^{ab}$ (4) | 0.026 $^{ab}$ (4) | 3.4 $^{b}$ (17) |
| T5: BGS + Biofertilizer | 2.82 a (4) | 0.68 $^{a}$ (41) | 1.59 $^{a}$ (6) | 0.027 $^{a}$ (9) | 4.3 $^{a}$ (49) |
| T6: EC + Biofertilizer | 2.81 ab (3) | 0.64 $^{ab}$ (33) | 1.57 $^{ab}$ (4) | 0.026 $^{ab}$ (5) | 4.0 $^{a}$ (37) |
| LSD Value | 0.0640 | 0.0775 | 0.0821 | 1.4520 | 0.5547 |
| CV | 1.30 | 7.47 | 2.98 | 3.19 | 8.98 |

**Table 6: Effect of biofertilizer in combination with organic amendments on growth parameters of maize**

| Treatment | Plant height (cm) | Root length (cm) | Shoots fresh biomass (g $pot^{-1}$) | Shoots dry biomass (g $pot^{-1}$) | Root fresh biomass (g $pot^{-1}$) | Root dry biomass (g $pot^{-1}$) | Number of leaves $plant^{-1}$ |
|---|---|---|---|---|---|---|---|
| T1: Control | 136 $^{f}$ | 12.25 $^{f}$ | 815 $^{f}$ | 195 $^{f}$ | 14.67 $^{e}$ | 3.00 $^{e}$ | 15.67 $^{b}$ |
| T2: Biogas Slurry (BGS) | 137 $^{d}$ (0.8) | 13.42 $^{d}$ (10) | 832 $^{d}$ (2) | 232 $^{d}$ (19) | 18.33 $^{c}$ (25) | 3.48 $^{c}$ (16) | 16.67 $^{ab}$ (6) |
| T3: Enriched Compost (EC) | 137 $^{e}$ (0.7) | 12.75 $^{e}$ (4) | 821$^{e}$(1) | 215 $^{e}$ (10) | 16.33 $^{d}$ (11) | 3.30 $^{d}$ (10) | 16.33 $^{ab}$ (4) |
| T4: Biofertilizer | 139 $^{c}$ (2.4) | 14.58 $^{c}$ (19) | 842 $^{c}$ (3) | 280 $^{c}$ (44) | 20.67 $^{b}$ (41) | 3.94 $^{b}$ (31) | 16.67 $^{ab}$ (6) |
| T5: BGS + Biofertilizer | 143 $^{a}$ (6) | 16.17 $^{a}$ (32) | 855 $^{a}$ (5) | 328 $^{a}$ (68) | 25.33 $^{a}$ (73) | 4.15 $^{a}$ (38) | 17.67 $^{a}$ (13) |
| T6: EC + Biofertilizer | 140 $^{b}$ (3) | 15.58 $^{b}$ (27) | 849 $^{b}$ (4) | 305 $^{b}$ (56) | 24.33 $^{a}$ (66) | 4.14 $^{a}$ (38) | 16.67 $^{ab}$ (6) |
| LSD Value | 2.7416 | 0.4569 | 3.3282 | 11.860 | 1.4525 | 0.0756 | 1.4525 |
| CV | 1.11 | 1.82 | 0.22 | 2.57 | 4.09 | 1.16 | 4.92 |

**Table 7: Effect of biofertilizer in combination with organic amendments on physiological and yield parameters of maize**

| Treatment | Relative water content (%) | Chlorophyll content (SPAD value) | Grain yield (g $cob^{-1}$) | 100 grain weight (g) | Stover yield $cob^{-1}$ (g) |
|---|---|---|---|---|---|
| T1: Control | 63.67 $^{e}$ | 29.99 $^{f}$ | 152 $^{f}$ | 32.8 $^{e}$ | 37.33 $^{e}$ |
| T2: Biogas Slurry (BGS) | 66.33 $^{cd}$ (4) | 38.30 $^{d}$ (28) | 160 $^{d}$ (5) | 34.0 $^{c}$ (4) | 43.67 $^{c}$ (17) |
| T3: Enriched Compost (EC) | 65.00 $^{de}$ (2) | 35.53 $^{e}$ (19) | 156 $^{e}$ (3) | 33.4 $^{d}$ (2) | 41.00 $^{d}$ (10) |
| T4: Biofertilizer | 67.67 $^{bc}$ (6) | 43.03 $^{c}$ (43) | 166 $^{c}$ (9) | 34.4 $^{b}$ (5) | 50.00 $^{b}$ (34) |
| T5: BGS + Biofertilizer | 70.33 $^{a}$ (10) | 49.55 $^{a}$ (65) | 172 $^{a}$ (14) | 35.0 $^{a}$ (7) | 54.33 $^{a}$ (46) |
| T6: EC + Biofertilizer | 69.33 $^{ab}$ (9) | 46.27 $^{b}$ (54) | 169 $^{b}$ (11) | 34.8 $^{a}$ (6) | 52.67 $^{a}$ (41) |
| LSD Value | 2.5848 | 2.3392 | 2.2882 | 0.03533 | 2.2188 |
| CV | 2.17 | 3.25 | 0.79 | 0.58 | 2.68 |

( ) indicates % increase from the control

*Means followed by the same letters are not statistically different at *$P<0.05$* according to the least significant difference (LSD) test

In our studies, application of biofertilizer along with biogas slurry improved the nodulation of chickpea in pot trial. In the rhizosphere, number of bacterial colonizes increased along the plant roots due to use of biofertilizer (Meunchang *et al.,* 2005; Ahmad *et al.,* 2014). It has been documented that in rhizosphere, growth promotion activities of rhizobacteria depend on effective colonization ability and survival in changing environment (Lugtenberg *et al.,* 2001). Plant growth and development is significantly affected by number of rhizobacteria (Frey-Klett *et al.,* 1999) and organic source present in rhizosphere.

Biofertilizers contain potential microbes that live in soil and fix atmospheric nitrogen along with some other traits beneficial for plant growth. These microbes form association with legumes to fix atmospheric nitrogen into plant useable form and that improves soil fertility status. This nitrogen not only used by the legume itself but also remains in soil for the next crop. Biogas slurry contains essential nutrients required for plant growth and improves soil fertility. Application of organic sources enhances organic matter in soil and improves soil physical properties. Many researchers studied that growing of legumes significantly improved yield of maize (McDonagh *et al.* 1993, Phoomthaisong *et al.* 2003). Increase in nodulation might be due to ability of chickpea to form symbiotic association with Rhizobium and the application of Rhizobium and biogas slurry (Togay *et al.*, 2008). This type of association between legume and Rhizobium is well documented and current studies also confirmed its significant role on yield and nodule formation in different legumes (Ahmad *et al.*, 2014). Moreover, improvement in nodulation might also be due to provision of additional infection sites by rhizobacteria for attachment of Rhizobia (Ahmad *et al.,* 2011).

due to individual as well as combined application of biofertilizer and organic sources were improved when biofertilizer was applied. It has been reported that there was a significant increase in leaf pigments and RWC after inoculation (Aslam *et al.*, 2011). Application of biofertilizer and organic sources improved all physiological attributes of plant (Vazin, 2012). The improvement in green pigments is due to the stabilization of chloroplasts and the scavenging ability of biofertilizers (Farooq *et al.*, 2009). Similar results were observed for improving growth, physiology and yield of maize under pot and field conditions due to rhizobium inoculation (Hussain *et al.,* 2014; 2016).

Combined application of biofertilizer and biogas slurry resulted in improvement in growth and yield parameters of chickpea and maize in pot trial. Biofertilizer improved the vegetative, reproductive growth and better fruit development (Samavat *et al.*, 2012; Ahmad *et al.,* 2013). The increase in growth might be attributed to many reasons including production of growth promoting hormones by the

**Table 8: Effect of biofertilizer in combination with organic amendments on grain quality and after harvesting parameters of maize**

| Treatment | N in grains (%) | P in grains (%) | K in grains (%) | Fe in grains (ppm) | Zn in grains (ppm) | Nitrogen in soil after harvesting (%) | Bacterial population after harvesting (cfu × $10^{-4}$) |
|---|---|---|---|---|---|---|---|
| Control | 2.36 [b] | 0.52 [e] | 2.80 [f] | 11.33 [e] | 11.33 [d] | 0.032 [c] | 4.25 [e] |
| Biogas Slurry (BGS) | 2.58 [b] (9) | 0.63 [d] (21) | 2.91 [d] (4) | 13.00 [c] (15) | 13.00 [bc] (15) | 0.032 [bc] (2) | 5.42 [cd] (27) |
| Enriched Compost (EC) | 2.54 [b] (7) | 0.60 [d] (15) | 2.86 [e] (2) | 12.00 [d] (6) | 12.33 [cd] (9) | 0.033 [bc] (3) | 5.00 [de] (18) |
| Biofertilizer | 2.63 [ab] (11) | 0.71 [c] (36) | 3.17 [c] (13) | 13.83 [b] (22) | 13.83 [ab] (22) | 0.034 [ab] (6) | 6.17 [bc] (45) |
| BGS + Biofertilizer | 3.04 [a] (29) | 0.80 [a] (53) | 3.31 [a] (18) | 15.50 [a] (37) | 14.83 [a] (31) | 0.035 [a] (9) | 7.33 [a] (73) |
| EC + Biofertilizer | 2.68 [ab] (13) | 0.75 [b] (45) | 3.24 [b] (16) | 15.17 [a] (34) | 14.17 [a] (25) | 0.034 [ab] (7) | 7.00 [ab] (65) |
| LSD Value | 2.4238 | 0.0368 | 0.0411 | 0.6290 | 1.0055 | 1.729 | 0.8895 |
| CV | 9.03 | 3.10 | 0.76 | 2.62 | 4.27 | 2.93 | 8.53 |

( ) indicates % increase from the control; *Means followed by the same letters are not statistically different at $P<0.05$ according to the least significant difference (LSD) test

Results of our study revealed that combined application of biofertilizer with biogas slurry significantly improved relative water contents, chlorophyll "a" and chlorophyll "b" that might be due to the increased root surface area and enhanced water uptake. However, improvement in relative water content was non-significant. These results are supported by the work of Ahmad *et al.* (2011) where they described that co-inoculation of PGPR and rhizobia increased the root length and improved water uptake from deeper soils. Amir *et al.* (2013) found that application of rhizobacteria improved stomatal conductance, root length and root surface area that increased water uptake from far places and resulted in improvement of relative water content. In present study, RWC and chlorophyll contents

*Pseudomonas* sp. that result in acceleration of cell division and enlargement. The nitrogen fixing Rhizobia also enhanced the nitrogen availability and the combined effect of Rhizobium and *Pseudomonas* sp. activity resulted in improved growth and better fruit development (Taiz and Zeigher, 2006; Ahmad *et al.,* 2013). The increased yield may also be due to the effect of phosphate solubilizing activity of *Pseudomonas* sp. that increased P uptake, leading to higher reproductive growth and flower development (Sánchez *et al.*, 2014).

Biogas slurry is preferred over other organic manures because it is already fermented and adds direct nutrients to the crop immediately after the application while the other organic sources need at least two to three weeks for the supply of nutrients to the crop plants. Due to quick response plants of biogas slurry after application to the soil, it increased the crop growth and production (Sanwal *et al.*, 2007). This might be due to the reason that PGPB increased the solubilization of nutrients (Hussain *et al.*, 2014; 2015; 2016), produced different plant growth promoting substances (direct) and siderophores (indirect) that suppress the pathogens with different mechanism of actions (Kloepper *et al.*, 1989). Biogas slurry is also helpful to increase the plant growth and yield as it contains higher concentration of essential plant nutrients as compared to other organic manures like farm yard manure, and provide carbon source to the soil microorganisms. The sole application of biogas slurry to the soil adds up organic matter and essential plant nutrients to the soil which aided in increasing the productivity of chickpea (Shahbaz *et al.*, 2014).

The nutritional analysis of maize and chickpea grains showed that the combined application of biofertilizer and biogas slurry resulted in increased uptake of N, P, K, Fe and Zn in pot trial. The rhizospheric microbial population also required these nutrients and their inoculation resulted in enhanced colonization of rhizobacteria that make available essential nutrients by solubilization and mineralization resulting in higher uptake by the plant and thus higher accumulation. The inoculation of Rhizobium increased the nodulation that resulted in increased supply of nitrogen to plant and also rhizospheric bacteria, and also increased their activity in rhizosphere (Ahmad *et al.*, 2011; 2013). The rhizospheric microbial population also required nutrients and their inoculation resulted in enhanced colonization of rhizobacteria that made available essential nutrients by mineralization and solubilization. A consistent approach to improve nutrient availability is to take benefits of the PGPR and their nutrients solubilizing ability (Illmer and Schinner, 1992). To do this, PGPR including *Pseudomonas* and Rhizobium can successfully be used for improving the nitrogen and phosphorus supply to crop plants (Halder *et al.*, 1990a; Alikhani *et al.*, 2006) that ultimately leads to enhanced yield and productivity of crop plants.

## Conclusion

The combined use of biofertilizer with biogas slurry was more effective in improving the growth, yield and yield contributing parameters in chickpea and maize crops than separate use of biogas slurry, biofertilizer and enriched compost. It can be concluded that the combined use of biofertilizer and biogas slurry is more efficient approach in improving the growth, physiology and yield in chickpea and maize crops that also improved soil bacterial population and soil health. The same may be evaluated under natural conditions for better productivity of chickpea-maize cropping system.

## Acknowledgement

Authors acknowledge the Department of Soil Science, University College of Agriculture and Environmental Sciences, The Islamia University of Bahawalpur, Pakistan for providing research facilities. We are also thankful to the Soil Microbiology and Biochemistry Laboratory and Environmental Sciences Laboratory at Institute of Soil & Environmental Sciences, University of Agriculture Faisalabad, Pakistan for provision of biofertilizer (Rhizogold) and P-enriched compost for experiments.

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