# Effectiveness of multi-strain biofertilizer in combination with organic sources for improving the productivity of Chickpea in Drought Ecology

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#### Abstract

The present study was conducted in Bahawalpur, which is characterized by extremely dry and harsh desert conditions with very little rainfall and water availability. Due to less availability of water, the production of food crops is unpredictable that is not enough to feed the local population. To cope with the shortage of cereal grains and water, it is need of the hour to arrange some alternate food sources that can be produced with limited water. Chickpea can be successfully grown under water limited conditions. Biofertilizers have the ability to improve the growth and nodulation of chickpea enables it to withstand the periods of drought. The study was involved evaluation of multi-strain biofertilizer (developed from novel strains of Mesorhizobium cicri, Pseudomonas sp. and Bacillus sp.) effectiveness in combination with organic sources for improving the productivity of chickpea in Cholistan desert area of Bahawalpur. Four field trials were conducted in different villages of Bahawalpur. Biofertilizer along with enriched compost and farmyard manure were applied under field conditions with three replications. The combined application of farm yard manure and biofertilizer improved nodules formation, plant growth, yield and chemical parameters as compared to control. It can be concluded that the use of biofertilizer in combination with farm yard manure is significantly effective in improving the productivity and profitability of chickpea. So, the farmers of Cholistan area should be recommended to adopt the biofertilizer technology along with organic manures that will not only enhance the grain yield but also rejuvenate the soil health.

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# Introduction

The Cholistan is a part of the world's seventh largest desert, stretched along the south border of Punjab province. The Cholistan has a fertile soil, but the nonavailability of irrigation water turned it into a desert. Moreover, frequent droughts resulting in a severe shortage of food in the Cholistan that is the main cause of hundreds of deaths in this area due to hunger. Pakistan is facing a famine-like situation in its arid climate districts i.e. Tharparkar and (Cholistan)



Bahawalpur. Due to the shortage of wheat grains and water scarcity, there is a need to find out some alternate ways and means to feed the undernourished people of the area.

Drought is a polygenic stress and is considered as one of the most important factors limiting crop production in desert areas. It adversely affects plant growth and yields that result in a shortage of food supply and the onset of famines in desert areas (Arshad et al., 2008). It has been reported that even a short-term water stress can cause significant losses in crop production (Pinheiro et al., 2005). However, the deleterious effects of water shortage on crop yield may be more obvious at some particular growth stage depending upon the nature of crop and species (Arshad et al., 2008).

Ethylene is the most important growth hormone, produced by almost all the plants under stress, which mediates a wide range of plant responses (Arshad and Frankenberger, 2002). The production of ethylene may be inhibitory or stimulatory depending upon its concentration, the nature of the physiological process and the growth phase of the plant. When it is present in high levels, it can be damaging to plants, leading to epinasty, shorter root, and premature senescence. The onset of drought may result in the production of the excess amount of ethylene, which causes inhibition of seed germination, root elongation and nodulation in legumes.

Chickpea has the potential to grow successfully under water limited conditions. It contributes more than 20% of the world pulse production (FAO, 2016). The growth of chickpea can be improved by using biofertilizer containing bacteria, which have the ability to improve plant growth through reducing the harmful effects of stresses. Under natural conditions, plants make symbiotic relationships with soil inhibiting plant growth promoting rhizobacteria (PGPR), rhizobia and fungi (Srinivasan et al., 1996). These play important role in helping the plants to withstand unfavorable conditions, like salinity (Ahmad et al., 2013; Joe et al., 2016), drought (Hussain et al., 2016), diseases (Nadeem et al., 2014), etc. In modern agriculture, these microorganisms are being used as biofertilizers i.e. live formulations of PRPR (O'Callaghan et al., 2001). The PGPR under drought conditions increases plant drought tolerance through different mechanisms. These mechanisms include the improvement in moisture availability to roots, enhanced nutrient availability, supplying phytohormones, and reduction in ethylene production (Zaheer et al., 2016). Different plant growth promoting compounds are supplied by bacteria through roots which alleviate the effect of stress on plants (Ahmad et al., 2011). They also facilitate the growth of their host plant by fixing atmospheric nitrogen, and siderophores production (which may solubilize and sequester iron thereby increasing its availability for plant uptake), producing phytohormones, and solubilizing minerals, such as phosphate, thus increasing their availability (Ahmad et al., 2011).

Keeping in view the entire scenario, it is imperative to find a technology that can easily be adapted by the resource poor farmers of the drought areas like the Cholistan. In our earlier studies at the University of Agriculture Faisalabad, we have developed a multistrain biofertilizer through a series of experiments under laboratory, green house, and field conditions. The biofertilizer developed from novel strains of Mesorhizobium cicri and PGPR (Bacillus and *Pseudomonas*) have the ability to improve the growth and nodulation of chickpea, thus enabling it to withstand the periods of drought. These fertilizers can be more effective for chickpea production in a desert climate. Using this biofertilizer is a practical, environment-friendly and sustainable approach which could consequently result in a better yield of chickpea. These biofertilizers can help in alleviating stress in plants grown in the arid and semi-arid environment (Ahmad et al., 2013). The present paper reports the results of experiments conducted on farmer's fields in Cholistan area of Bahawalpur to evaluate the use of biofertilizer for chickpea production under limited water resources.

## Materials and Methods

#### **Preparation of biofertilizer**

A multi-strain biofertilizer containing novel strains of *Mesorhizobium cicri*, *Pseudomonas* sp. and *Bacillus* sp. was prepared at the University of Agriculture Faisalabad. The already installed biofertilizer production unit was used to prepare the bio-fertilizer, which was used to inoculate the seed for field trials.

#### Sowing of crop in the field

Four sites at different villages in Cholistan area were selected and sowing of chickpea crop was conducted in November 2014. The treatments comprised of control, farmyard manure, P-enriched compost, bio fertilizer, farmyard manure + biofertilizer, and P-

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enriched compost + bio fertilizer. The experiments were conducted in a Randomized Complete Block design (RCBD) with three replications. Recommended dose of P and K @ 60 kg ha<sup>-1</sup> each plot while half of the recommended dose of nitrogen (20 kg ha<sup>-1</sup>) was applied as basal dose and seeds of chickpea were inoculated with bio-fertilizer. Sowing was done by hand drill and row to row distance was maintained 30 cm, while plant to plant distance 5-7 cm. All agronomic practices were adopted during the growth period of the crop.

#### Data recording and plant analysis

Nodulation data were recorded at flowering by carefully uprooting the plants with enough surrounding soil, keeping in view that minimal damage to roots occurs. Five plants per experimental unit were uprooted along with rhizospheric soil, washed thoroughly under running water on a sieve of mesh size that doesn't allow nodules to pass through but the soil. Nodules were counted and fresh as well as dry weight was recorded. At harvesting, plant agronomic data were recorded and samples were collected for analyses. Dried ground plant material was digested according to the method of Wolf (1982) and N, P, and K concentration was determined as described by Ryan et al. (2001).

Physiological parameters i.e. chlorophyll-a, and chlorophyll-b contents in leaves were recorded at physiological maturity by extracting leaf material (0.05 g) in 10 mL dimethyl sulfoxide (Hiscox and Israelstam, 1979). The samples were heated at 65°C for 4 h and then the absorbance of the extract was recorded at 663 and 645 nm as described by Arnon (1949). Data were subjected to statistical analysis by using LSD technique at 5% level of significance (Steel et al., 1997).

## Results

Results of the experiments conducted at four locations in district Bahawalpur are summarized in this section. It has been observed that separate application of biofertilizer, farm yard manure, and compost efficiently improved the growth and yield in chickpea in all trials. However, the integrated use of biofertilizer with farm yard manure and enriched compost was more effective in improving all the parameters as compared to the individual use of these sources. It is obvious in *Figure 1*, where clear difference among treatments in the improvement of root growth of chickpea can be noticed.

The data given in table 1 depicts that combined use of biofertilizer with farmyard manure and enriched compost significantly increased the number of nodules. Among sole applications, the biofertilizer resulted in a maximum increase in a number of nodules followed by farmyard manure and enriched compost. The combined application of biofertilizer with farmyard manure gave 3 fold more nodules than control plots from an experiment at 138DB while the combination biofertilizer with enriched compost resulted in 227% increase in a number of nodules at experimental site Jageer bhati dari. The sole application of biofertilizer also gave better results as compared to the sole application of farmyard manure and enriched compost. The combination of biofertilizer with farm yard manure gave 195% more nodules fresh weight when compared with control from the experiment at Baghdad research farm.

The results regarding plant height (Table 2) showed that the combined use of biofertilizer with farmyard manure and enriched compost significantly increased plant height. However, there was no significant difference among the sole application of farmyard manure and enriched compost. The maximum increase in plant height occurred with combined application of enriched compost and biofertilizer followed by farmyard manure and biofertilizer. The results regarding shoot dry weight (Table 2) showed that the use of organic nutrient sources and biofertilizer, individually and in combination, significantly enhanced shoot dry weight of chickpea. The sole application of farmyard manure, enriched compost and biofertilizer significantly increased shoot dry weight in chickpea, though the combined application gave better results. The maximum increase in shoot dry weight was observed in the treatment where biofertilizer was integrated with farmyard manure.

The results given in Table-3 showed that the number of pods plant<sup>-1</sup> increased significantly by the application of biofertilizer, farmyard manure, and enriched compost. The sole application of biofertilizer showed significantly better results than sole application of farmyard manure and enriched compost and. While, combined application of biofertilizer with farmyard manure and enriched compost showed a maximum increase in a number of pods plant<sup>-1</sup>, which is significantly higher than individual application of bio fertilizer, farmyard manure, and enriched compost. The results in Table-3 also showed that biofertilizer

with farmyard manure and enriched compost significantly increased the number of grains pod<sup>-1</sup> of chickpea. A significant increase in grains pod<sup>-1</sup> was recorded with combined application of farmyard manure and biofertilizer followed by the combined use of enriched compost and bio fertilizer.

The data showed that the use of biofertilizer with farmyard manure and enriched compost significantly increased 1000 grains weight in chickpea (Table 4). The maximum increase in 1000 grains weight was recorded with combined application of farmyard manure and biofertilizer followed by enriched compost + biofertilizer and sole application of biofertilizer. The grain yield was also significantly increased with separate as well as combined application of biofertilizer and organic sources. At all experimental sites, maximum increase in grain yield was observed where biofertilizer and farmyard manure were applied in combination (Table 4).

The application of organic nutrient sources and biofertilizer, individually and in combination, significantly improved the chlorophyll-a and chlorophyll-b contents in leaves of chickpea (Table 5).The combined application of biofertilizer along with farmyard manure and enriched compost showed maximum increase in chlorophyll-a and chlorophyll-b contents in leaves of chickpea. The chemical analysis of grains showed that nitrogen concentration in grains significantly increased by the application of biofertilizer in combination with farmyard manure and enriched compost. The results showed that sole application of farmyard manure, biofertilizer, and enriched compost showed non-significant difference regarding nitrogen concentration in grains (Table 6). However, combined application of biofertilizer with farmyard manure and enriched compost resulted in significant increase in nitrogen concentration in grains of chickpea.

The data regarding phosphorus concentration (Table 6) showed that the integrated use of organic nutrients and biofertilizer significantly increased phosphorus concentration in grains of chickpea. Sole application of farmyard manure, enriched compost, and biofertilizer significantly increased phosphorus concentration in grains as well. It was observed that increase in phosphorus in grains was significantly higher when biofertilizer was applied solely, as compared to the sole application of farmyard manure and enriched compost. Maximum increase was recorded when biofertilizer was applied in combination with farmyard manure. The results showed that there was the non-significant effect of farmyard manure, enriched compost and biofertilizer on potassium uptake (Table 7). No significant increase in potassium concentration in grains of chickpea was observed among all the treatments at all the experimental sites.

 Table - 1: Effect of farmyard manure, enriched compost and biofertilizer on number of nodules plant<sup>-1</sup>

 and nodules fresh weight of chickpea

		No. of nodules plant <sup>-1</sup>				Nodules fresh weight (g plant <sup>-1</sup> )			
Treatment	107 DB	Jageer bhati dari	138DB	Baghdad	107 DB	Jageer bhati dari	138DB	Baghdad	
Control	31 e	22 f	19 f	25 f	0.65 e	0.42 f	0.41 f	0.55 e	
FYM	49 d	36 d	32 d	41 d	0.94 c	0.81 d	0.78 d	0.85 c	
Enriched compost (EC)	37 e	27 e	25 e	31 e	0.78 d	0.62 e	0.54 e	0.67 d	
<b>Biofertilizer (B)</b>	55 c	55 c	51 c	59 c	1.27 b	0.98 c	0.91 c	1.16 b	
FYM + B	76 a	82 a	79 a	86 a	1.77 a	1.65 a	1.59 a	1.62 a	
EC + B	62 b	72 b	61 b	77 b	1.53 b	1.26 b	1.12 b	1.41 b	
<b>LSD</b> (p < 0.05)	5.6139	3.5722	3.3724	5.1245	0.1448	0.0770	0.0976	0.1342	

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

	Plant height (cm)				Shoot dry weight (g)			
Treatment	107 DB	Jageer bhati dari	138DB	Baghdad	107 DB	Jageer bhati dari	138DB	Baghdad
Control	45.58 c	50.29 d	38.98 d	49.39 c	12.30 e	14.43 e	11.03 d	13.86 f
FYM	49.85 b	54.54 c	44.54 c	54.03 b	16.16 c	17.26 cd	14.01 c	16.99 d
Enriched compost (EC)	48.88 b	53.73 c	43.14 c	53.58 b	15.02 b	15.94 e	12.97 c	15.94 e
<b>Biofertilizer</b> (B)	54.99 a	58.69 b	47.70 b	57.78 a	18.43 b	18.19 c	16.49 b	18.19 c
FYM + B	56.34 a	61.34 a	49.60 a	59.50 a	20.07 a	20.26 a	19.38 a	20.26 a
EC + B	56.99 a	60.83 a	50.53 a	59.28 a	18.52 b	19.68 b	18.14 a	19.68 b
<b>LSD</b> (p < 0.05)	1.8632	1.9390	1.6158	1.9182	0.7964	0.5786	1.4025	0.5786

 Table - 2: Effect of farmyard manure, enriched compost and biofertilizer on plant height and shoot dry weight of chickpea

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

Table - 3: Effect of farmyard manure (FYM), enriched compost and biofertilizer on number of pods
plant <sup>-1</sup> and number of grains pod <sup>-1</sup> of chickpea

	Number of pods plant <sup>-1</sup>				Number of grains pod <sup>-1</sup>			
Treatment 10	107 DB	Jageer bhati dari	138DB	Baghdad	107 DB	Jageer bhati dari	138DB	Baghdad
Control	29.69 e	33.42 d	31.71 e	33.79 d	1.42 c	1.46 d	1.16 d	1.39 d
FYM	35.58 c	36.05 c	37.16 c	38.53 c	1.47 b	1.52 c	1.27 bc	1.48 c
Enriched compost (EC)	33.69 d	34.71 cd	35.80 d	37.94 c	1.50 b	1.49 cd	1.24 c	1.45 c
<b>Biofertilizer</b> (B)	39.97 b	41.25 b	39.14 b	43.98 b	1.56 a	1.56 d	1.29 ab	1.53 b
FYM + B	44.12 a	43.93 a	42.54 a	49.86 a	1.59 a	1.61 a	1.32 a	1.61 a
EC + B	40.68 b	42.96 a	39.56 b	45.05 b	1.59 a	1.60 ab	1.25 c	1.58 a
<b>LSD</b> (p < 0.05)	1.3005	1.4836	0.83 56	1.3532	0.0354	0.0345	0.0348	0.0380

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

Table - 4: Effect of farmyard manure (FYM), enriched compost and biofertilizer on 1000 grains weigh	t
and grain yield of chickpea	

	1000 grains weight (g)				Grain yield (kg acre <sup>-1</sup> )			
Treatment	107 DB	Jageer bhati dari	138DB	Baghdad	107 DB	Jageer bhati dari	138DB	Baghdad
Control	255.00 e	256.33 d	230.00 d	274.67 e	881.70 d	821.7 e	848.3 c	715.0 d
FYM	259.00 d	258.33 cd	235.33 c	278.00 d	967.70 b-d	1011.0 bc	997.0 b	1011.0 a-c
Enriched compost (EC)	261.33 c	260.67 bc	237.67 b	280.33 c	930.30 cd	911.3 b	827.7 c	897.0 c
Biofertilizer (B)	263.67 b	262.67 ab	240.33 a	283.67 b	1047.0 a-c	963.7 cd	897.0 bc	957.0 bc
FYM + B	266.00 a	265.00 a	242.00 a	285.67 ab	1162.3 a	1162.3 a	1180.3 a	1129.0 a
EC + B	264.67 ab	265.00 a	241.33 a	287.67 a	1071.3 ab	1022.7 b	927.7 bc	1046.0 ab
<b>LSD</b> (p < 0.05)	2.1440	2.8379	1.6828	2.0204	118.35	58.968	128.51	138.41

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

		Chlorophy	ll-a (µg g <sup>-1</sup>	)	Chlorophyll-b (µg g <sup>-1</sup> )				
Treatment	107 DB	Jageer bhati dari	138DB	Baghdad	107 DB	Jageer bhati dari	138DB	Baghdad	
Control	1.326 c	1.313 d	1.306 c	1.323 c	0.710 c	0.613 e	0.676 c	0.796 d	
FYM	1.373 b	1.356 c	1.343 b	1.373 b	0.756 a-c	0.640 de	0.713 b	0.843 bc	
Enriched compost (EC)	1.406 b	1.360 c	1.320 bc	1.370 b	0.746 a-c	0.656 cd	0.696 a-c	0.826 cd	
<b>Biofertilizer</b> (B)	1.466 a	1.436 b	1.390 a	1.440 a	0.786 ab	0.680 bc	0.753 a	0.866 ab	
FYM + B	1.493 a	1.500 a	1.406 a	1.446 a	0.800 a	0.723 a	0.770 a	0.890 a	
EC + B	1.496 a	1.506 a	1.390 a	1.443 a	0.730 bc	0.706 ab	0.763 a	0.873 ab	
<b>LSD</b> (p < 0.05)	0.0354	0.0224	0.0289	0.0394	0.0671	0.0268	0.0248	0.0315	

 Table - 5: Effect of farmyard manure (FYM), enriched compost and biofertilizer on chlorophyll-a and chlorophyll-b contents in leaves of chickpea

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

Table - 6: Effect of farmyard manure (FYM),	enriched compost and biofertilizer on nitrogen and
phosphorus concentration in grains of chickpea	

	Nitrogen (%)				Phosphorus (%)			
Treatment	107 DB	Jageer bhati dari	138DB	Baghdad	107 DB	Jageer bhati dari	138DB	Baghdad
Control	1.68 b	1.68 c	1.61 c	1.48 c	0.45 c	0.38 c	0.72 c	0.59 c
FYM	1.87 ab	1.87 bc	1.84 b	1.77 bc	0.50 bc	0.40 bc	0.77 bc	0.61 bc
Enriched compost (EC)	1.85 ab	1.85 bc	1.76 bc	1.69 bc	0.50 bc	0.47 a	0.77bc	0.67 ab
Biofertilizer (B)	1.99 ab	1.99 b	1.92 b	1.86 b	0.55 ab	0.44 ab	0.79 a-c	0.69 a
FYM + B	2.22 ab	2.20 a	2.15 a	2.05 a	0.51 bc	0.49 a	0.85 a	0.71 a
EC + B	2.11 a	2.08 a	1.97 ab	1.84 b	0.58 a	0.48 a	0.83 ab	0.73 a
<b>LSD</b> (p < 0.05)	0.4539	0.2473	0.2110	0.1911	0.0690	0.0586	0.0771	0.0721

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

Table - 7: Effect of farmyard manure (FY)	M), enriched compost and biofertilizer on potassium
concentration in grains and nodules dry weight of	f chickpea

	Potassium (%)				Nodules dry weight (g plant <sup>-1</sup> )			
Treatment	107 DB	Jageer bhati dari	138DB	Baghdad	107 DB	Jageer bhati dari	138DB	Baghdad
Control	1.50 a	1.53 a	1.72 a	1.78 b	0.15 f	0.12 f	0.17 f	0.11 f
FYM	1.53 a	1.56 a	1.76 a	1.80 ab	0.19 d	0.16 d	0.20 d	0.17 d
Enriched compost (EC)	1.54 a	1.57 a	1.75 a	1.82 ab	0.16 e	0.14 e	0.18 e	0.13 e
<b>Biofertilizer</b> (B)	1.56 a	1.61 a	1.78 a	1.84 a	0.18 c	0.21 c	0.20 c	0.19 c
FYM + B	1.57 a	1.63 a	1.80 a	1.86 a	0.26 b	0.24 a	0.28 b	0.23 a
EC + B	1.59 a	1.63 a	1.79 a	1.86 a	0.23 a	0.22 b	0.25 a	0.20 b
<b>LSD</b> (p < 0.05)	0.0904	0.1069	0.0525	0.0912	0.0240	0.0393	0.0194	0.0263

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



Figure – 1: Effect of farmyard manure, enriched compost and biofertilizer on nodulation in chickpea E. Compost: P-enriched Compost, FYM: Farmyard Manure, B: Biofertilizer

#### Discussion

The results of our study showed that the inoculation with multi-strain biofertilizer containing Mesorhizobium cicri, Pseudomonas sp. and Bacillus sp. significantly improved the nodulation of chickpea at all experimental sites. It might be due to indirect stimulation in nodulation by rhizobacteria through increased root growth that provided more infection sites to rhizobia for nodulation. The improvement in nodule occupancy by Bradyrhizobium in soybean has also been reported by Mishra et al. (2009) due to coinoculation with P. fluorescens. This kind of positive legume-Rhizobium association is well documented and recent reports confirmed its positive role on yield and nodulation of different legumes (Ahmad et al., 2013). The legume-Rhizobium association brought a number of changes in legumes but nodulation is the most important, as it has a central position in their developmental growth stages through N2- fixation from the atmosphere.

Lowest nodulation in un-inoculated control elucidates decreased the activity of native rhizobia but the addition of organic nutrient sources (enriched compost and farmyard manure) and Rhizobium individually and in combination increased nodulation. It is advocated that the application of farmyard manure and enriched compost resulted in increased Rhizobium bioactivity in the rhizosphere and increased the colonization of bacteria that promoted the nodulation and also these organic sources enhanced the carbon and nutrient availability for the proliferation of rhizospheric Rhizobium (Zahran, 1999; Otieno et al., 2007). Organic amendments also boost nutrient availability (by solubilization and dissolution) ensuring plant uptake with enhancement of plant growth as well as nodulation (Bashan et al., 2013). The results of the study under discussion showed that use of biofertilizer, enriched compost, and farmyard manure significantly increased yield attributes of chickpea. The findings were in line with the studies of Gharib et al. (2008) who reported that the use of biofertilizer and enriched compost enhanced crop yield significantly by improving the grain weight and their numbers. In addition to the positive attributes of organic sources, the application of biofertilizer enhanced nutrient uptake and growth leading to improved yield and grain nutritional quality. The increase in yield most likely due to the promotion of root growth by the decreased ethylene levels due to ACC-deaminase activity of rhizobacteria present in multi-strain biofertilizer (Ahmad et al., 2012) along with some other growth promoting attributes. The increase in growth and yield components of chickpea by combined application of biofertilizer and organic amendments may also be attributed to cumulative

effects, such as enhanced supply of nitrogen and phosphorus to the crop and other growth promoting substances produced by microorganisms present in biofertilizer (Ahmad et al., 2013). In addition to biocontrol activity against soil born fungal pathogens, PGPR also increased grain yield by suppressing the diseases (Vinale et al., 2008). So, the reduced activity of pathogens and disease incidences may also be attributed to increased grain yield to.

The increase in growth and yield of chickpea could also be related to nutrient supplementation by the bacteria present in multi-strain biofertilizer and the role of organic fertilizers in promoting the colonization of these microbes (Ahmad et al., 2014), which might have enhanced their efficiencies like N<sub>2</sub> fixation, P-solubilization, and effective pathogen suppression. Similarly, increasing nitrogen fixation due to phosphorus supplementation has been reported by Manjunath and Bagyaraj (1984).

The present study demonstrated the effectiveness of biofertilizer in combination with enriched compost and farmyard manure for improving growth, yield and nutritional quality (N, P, and K) of chickpea both individually and in combination at different experimental sites in Cholistan villages. The increase in grain quality could be attributed to the enhanced nutrient use efficiency in the presence of organic fertilizers and bio-inoculants. Studies have shown that the composted organic materials release nutrients slowly and may reduce the leaching losses, particularly of N (Muneshwar et al., 2001) and thus enhance nutrient use efficiency (Rudresh et al., 2005). This premise is further supported by the fact that total N and P uptakes in chickpea were significantly increased in response to combined application of biofertilizer and organic fertilizers as P-enriched compost and farmyard manure increase the solution P, and also phosphate solubilizing action of inoculants and nitrogen fixing ability of bacterial strains enhancing the nutrients uptake (Ahmad et al., 2014). All these factors resulted in an enhanced nutrient uptake leading to better quality grains (Nevens and Reheul, 2003). The increase in nutrient uptake may be due to an increase in available N and P contents in the soil, and improved soil structure due to increased organic matter that increased nutrients availability and hence enhanced growth and quality of the crop (Manna et al., 2001).

# Conclusion

In the present study, the application of biofertilizer, farmyard manure, and enriched compost significantly enhanced the nodulation, growth, yield, grain quality and physiology of chickpea under field conditions. It is concluded that the use of biofertilizer in combination with organic nutrient sources is significantly effective in improving the productivity and profitability of chickpea. The combined use of biofertilizer in combination with farm yard manure was the most effective treatment. So, the farmers of the desert and drought areas like the Cholistan should be recommended to adopt the biofertilizer technology along with organic manures to get a higher yield of chickpea.

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## References

- Ahmad M, Zahir ZA, Jamil M, Nazli F, Latif M and Akhtar MF, 2014. Integrated use of plant growth promoting rhizobacteria, biogas slurry and chemical nitrogen for sustainable production of maize under salt-affected conditions. Pak. J. Bot. 46(1): 375-382.
- Ahmad I, Akhtar MJ, Asghar HN and Khalid M, 2013. Influence of Rhizobium applied in combination with micro nutrients on mung bean. Pak. J. Life Social Sci. 11:53-59.
- Ahmad M, Zahir ZA, Nadeem SM, Nazli F, Jamil M and Khalid M, 2013. Field evaluation of *Rhizobium* and *Pseudomonas* strains to improve growth, nodulation, and yield of mung bean under salt-affected conditions. Soil Environ. 32(2):

- Ahmad M, Zahir ZA, Asghar HN and Arshad M, 2012. The combined application of rhizobial strains and plant growth promoting rhizobacteria improves growth and productivity of mung bean (*Vigna radiata* L.) under salt-stressed conditions. Ann. Microbiol. 62: 1321–1330
- Ahmad M, Zahir ZA, Asghar HN and Asghar M, 2011.
   Inducing salt tolerance in mung bean through coinoculation with *Rhizobium* and PGPR containing ACC-deaminase. Can. J. Microbiol. 57(7): 578-589
- Arnon DI, 1949. Copper enzymes in isolated chloroplasts, polyphenoxidase in *Beta vulgaris*. Plant Physiol. 24: 1-15.
- Arshad M and Frankenberger Jr. WT, 2002. Ethylene: Agriculture Sources and Applications. Kluwer Academic Publishers, New York, USA.
- Arshad M, Shaharoona B and Mahmood T, 2008. Inoculation with *Pseudomonas* spp. containing ACC-deaminase partially eliminates the effects of drought stress on growth, yield, and ripening of pea (*Pisum sativum* L.). Pedosphere. 18: 611-620.
- Bashan Y, Kamnev AA and de-Bashan LE, 2013. A proposal for isolating and testing phosphatesolubilizing bacteria that enhance plant growth. Biol. Fertil. Soils. 49: 1-2.
- FAO, 2015. FAOSTAT. Food and Agriculture Organization of the United Nations. http://faostat.fao.org/site/291/default.aspx.
- Gharib FA, Moussa LA, and Massoud ON, 2008. Effect of compost and bio-fertilizers on growth, yield and essential oil of sweet marjoram (*Majorana hortensis*) plant. Int. J. Agri. & Biol. 10: 381-387.
- Hiscox JD and Israelstam GF, 1979. A method for the extraction of chlorophyll from leaf tissue without maceration. Can. J. Microbiol. 57: 1332-1334
- Hussain MB, Zahir ZA, Asghar HN, Mubaraka R and Naveed M, 2016. Efficacy of rhizobia for improving photosynthesis, productivity, and mineral nutrition of maize. Clean Soil, Air, Water. 44(11): 1564-1571
- Joe MM, Devaraj, S, Benson A and Sa T, 2016. Isolation of phosphate solubilizing endophytic bacteria from *Phyllanthus amarus* Schum & Thonn: Evaluation of plant growth promotion and antioxidant activity under salt stress. J. Appl. Res. Medicin. Arom. Plants. 3: 71-77.

- Manjunath A and Bagyaraj DJ, 1984. The response of pigeon pea and cowpea to phosphorus and dual inoculation with vesicular arbuscular Mycorrhiza and Rhizobium. Tropical Agriculture. 61: 48-52.
- Manna MC, Ghosh PK, Ghosh BN and Singh KN, 2001. Comparative effectiveness of phosphateenriched compost and single super- phosphate on yield, uptake of nutrients and soil quality under soybean–wheat rotation. J. Agri. Sci. 137: 45–54.
- Mishra PK, Mishra S, Selvakumar G, Kundu S and Shankar GH, 2009. Enhanced soybean (*Glycine* max L.) plant growth and nodulation by *Bradyrhizobium japonicum*-SB1 in presence of *Bacillus Thuringian is*. Acta. Agr. Scand. B-S. P. 59:189-196.
- Singh M, Singh VP, and Reddy KS, 2001. Effect of integrated use of fertilizer nitrogen and farmyard manure or green manure on the transformation of N, K and S and productivity of rice-wheat system on a vertisol. J. Society Soil Sci. 49: 430-435
- Nadeem SM, Ahmad M, Zahir Z A, Javaid A and Ashraf M, 2014. The role of mycorrhizae and plant growth promoting rhizobacteria (PGPR) in improving crop productivity under stressful environments. Biotechnol. Adv. 32: 429-448.
- Nevens F and Reheul D, 2003. The application of vegetable, fruit, and garden waste (VFG) compost in addition to cattle slurry in a silage maize monoculture: nitrogen availability and use. Eur. J. Agron. 19: 189-203.
- O'Callaghan KJ, Dixon RA and Cocking EC, 2001. *Arabidopsis thaliana*: a model for studies of colonization by non-pathogenic and plant-growthpromoting rhizobacteria. Funct. Plant Biol. 28: 975-982.
- Otieno PE, Muthomi JW, Cheminingwa GN and Nderitu JH, 2007. Effect of rhizobia inoculation, farmyard manure and nitrogen fertilizer on growth, nodulation, and yield of selected food grain legumes. In African Crop Science Conference Proceedings. 8:305-312.
- Rudresh DL, Shivaprakash MK and Prasad RD, 2005. Effect of combined application of Rhizobium, phosphate solubilizing bacterium and *Trichoderma* spp. on growth, nutrient uptake and yield of chickpea (*Cicer aritenium* L.). Appl. Soil Ecol. 28: 139-146

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- Ryan J, Estefan G and Rashid A, 2001. Soil and Plant Analysis Laboratory Manual, 2nd Ed. International Center for Agriculture in Dry Areas (ICARDA), Syria
- Srinivasan M, Holl FB and Petersen DJ, 1996. Influence of indole acetic-acid-producing *Bacillus* isolates on the nodulation of *Phaseolus vulgaris* by *Rhizobiumetli* under gnotobiotic conditions. Can. J. Microbiol. 42(10): 1006-1014
- Steel RGD, Torrie JH and Dickey DA, 1997. Principles and Procedures of Statistics: A Biometrical Approach. 3<sup>rd</sup> Ed. WCB/McGraw-Hill, Boston, Mass., USA
- Vinale F, Sivasithamparam K, Ghisalberti EL, Marra R, Woo SL and Lorito M, 2008. Trichodermaplant-pathogen interactions. Soil Biol. Biochem. 40: 1-10
- Wolf B, 1982. The comprehensive system of leaf analysis and its use for diagnosing crop nutrient status. Commun. Soil Sci. Plan. 13:1035-1059
- Zaheer A, Mirza BS, Mclean JE, Yasmin S, Shah TM, Malik KA and Mirza MS, 2016. Association of plant growth-promoting Serratia spp. with the root nodules of chickpea. Res. Microbiol. 167: 510-52.

