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Yield Response of *Cicer arientium* L. as Influenced by Plant Growth Promoting Rhizobacteria in Rainfed Loess Soils

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Authors' contributions

This work was carried out in collaboration between all authors. Authors MR and OUR designed the study. Authors MR, SMM, SA and AAS performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors OUR and SMM managed the analyses of the study. Authors HM, MIA and RAR managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Climate change has greatly affected rainfall distribution and intensity in the rainfed areas of the world which has created water shortage and soil moisture deficiency and causing crop yield reduction. Soil microorganisms have great potential to improve soil fertility and enhance plant nutrition by minimizing the damages of water stress since water stress inhibits plant growth due to higher concentrations of ethylene in rhizosphere. Rhizobacteria containing ACC-deaminase, assist plant growth to tolerate these injurious effects. To test this hypothesis under rainfed sloppy lands an experiment was conducted consisting of treatments including gypsum, plant growth promoting

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rhizobacteria (PGPR), farmyard manure (FYM) and, PGPR+FYM in randomized complete block design (RCBD) arrangement to assess their effectiveness for improving growth of chickpea and changes in soil characteristics. The results of this experiment revealed that selected amendments increased grain and straw yield significantly ($p \leq 0.05$). The mean maximum grain yield (1519 Kg ha-1) and biomass yield (1862 Kg ha-1) was recorded where PGPR was applied in combination with FYM. It was observed that grain yield was enhanced 6, 11, 13 and 19 % and biomass yield was increased 12, 15, 17 and 23% by gypsum, PGPR, FYM and PGPR+FYM, respectively over control (zero addition). Application of amendments enhanced soil organic matter and saturation percentage up to 27 and 5%, respectively while soil pH and EC were reduced by 2 and 18%, respectively. So, it was concluded that soil amendments especially PGPR can be used effectively to combat the soil moisture shortage under stress conditions to enhance the soil and crop productivity in rainfed conditions under changing climate scenario.

Keywords: PGPR; soil characteristics; soil fertility; rain fed; Pakistan.

1. INTRODUCTION

Plants growth is confronted with many biotic and abiotic stresses. These stresses include extremes of temperature, flooding, drought, disease, insects, soluble salts, toxic metals and environmental organic contaminants [1]. Plant has to negotiate these limitations to regulate its metabolism to overcome the stresses otherwise plant growth would be inferior. Under this stress period, plants release ethylene hormone which indicate multifarious physiological changes in plants at molecular level. Ethylene is, apart from plant growth regulator, also a stress hormone. It is accelerated considerably under stress situation like those generated by salinity, drought, and pathogenicity etc [2]. In an apparent paradox, ethylene has been suggested to both alleviate and exacerbate the effects of stress [3]. The rhizosphere is a complex location where roots interact with physical, chemical and biological properties of soil. Structural and functional characteristics of roots contribute to rhizosphere processes and both have significant influence on the capacity of roots to acquire nutrients. Roots also interact extensively with soil microorganisms which further impact on plant nutrition either directly, by influencing nutrient availability and uptake, or indirectly through plant (root) growth promotion [4]. Plants transform the physico-chemical properties and biological composition of rhizosphere through a range of mechanisms, which include acidification through proton extrusion and the release of root exudates. Along with changes to soil pH, root exudates directly influence nutrient availability or have indirect effects through interaction with soil microorganisms [5].

Water availability is the major limitation to crop yields in the rainfed regions all over the world [6]. As the growing season progresses and surface soil water is depleted, much of the water and nutrient supply function of the roots is determined by a relatively small proportion of roots that are at the leading edge of root system development. The rate of progress of the root system down the soil profile is therefore an important determinant of yield [7]. Soil nutrients arrested by roots is an important phenomena often improved by the application of fertilizers. Availability of nutrients is governed by a range of physico-chemical, environmental, seasonal and biological interactions [8]. Microbial associations with roots are complex in soil and can enhance the ability of plants to acquire nutrients from soil through a number of mechanisms [9]. The overproduction of ethylene in response to abiotic and biotic stresses leads to inhibition of root growth and consequently growth of the plant as a whole. Ethylene synthesis is stimulated by a variety of environmental factors/stresses, which hamper plant growth. PGPR (plant growth promoting rhizobacteria) containing ACC deaminase regulates and lowers the levels of ethylene by metabolizing ACC; a precursor of plant produced ethylene. PGPR containing ACC deaminase, boost plant growth particularly under stressed conditions by the regulation of accelerated ethylene production in response to a multitude of abiotic and biotic stresses. It is very likely that root growth and nodulation could be affected due to production of higher levels of ethylene in roots under field conditions. Since the bacterial enzyme ACC-deaminase lowers the level of ethylene in roots, inoculation with PGPR containing ACC-deaminase could be very effective in improving growth and nodulation of chickpea. The use of rhizobacteria containing ACC-deaminase in co-inoculation with rhizobia could be more beneficial due to their multiple effects on plant through different growth enhancing mechanisms. Application of humus like organic material in the soil could provide a niche that might have positive effect on plant growth most likely by supporting activities of PGPR in the rhizosphere of a plant throughout its life cycle [10,11]. Implementation of PGPR-based biofertilizer technology presents economic, environmental and agronomic benefits and could be used to a larger degree to partially replace the synthetic fertilizers [12] to improve economic yield under natural conditions.

Pothwar rainfed plateau of Pakistan is featured by its uneven land terrain. Torrential Monsoon rains generate a great run off results in loss of fertile soil top layer along with essential soil nutrients. Rehabilitation of these degraded lands requires a comprehensive nutrient management strategy to develop soil fertility and soil structure which may resist moisture loss and fertilizer use efficiency. Current climate change projections indicate that dry conditions are likely to intensify in these climatic regions [13] requiring farming systems to develop strategies that increase their resilience and prepare for potential shortages of irrigation water. Chickpea is one of the most important dry land field crops. Its' per hectare yield in Pakistan is much lower than its yield potential, because of suboptimal growth conditions faced by this crop during different growth stages and chemical fertilizers are the most important input required for chickpea cultivation. In order to make its cultivation sustainable and less dependent on chemical fertilizers, it is important to know the use PGPR that can biologically fix nitrogen, solubilize phosphorus and induce some substances like Indole Acetic Acid (IAA) that could contribute to the improvement of chickpea growth. Thus the aim of this study was to determine the effect of plant growth promoting rhizobacteria for improvement of germination and plant Growth of chickpea (*Cicer arietinum* L.).

2. MATERIALS AND METHODS

2.1 Location, Climate and Soils

This experiment was conducted in research area of SAWCRS, Fateh Jang (latitude 33.55° N, longitude 72.58°E and 402 m high from the sea level) Pakistan. It is situated on sloppy terraced lands prone to gully development. The location of this experiment have semi-arid climate with average annual rainfall 700-1000 mm. Most of rainfall received in summer and only around 30% is received during the gram growing season (October-April). The soils of the study areas are categorized as moderately deep, well drained, medium textured, calcareous loess deposited [14].

2.2 Experimental Setup

The study was conducted during 2010/11 to 2013/14 during rabbi at SAWCRS, Fateh Jang. Chickpea (*Cicer arientium* L.) was grown with consisting of PGPR, FYM, FYM+PGPR and Gypsum along with Control (zero addition). The experiment was laid in randomized complete block design (RCBD) with 3 replications. All the treatments were replicated thrice. PGPR inoculum was applied prior to sowing as seed application. FYM @ 300 and Gypsum @ 1000 Kg ha^{-1} was applied.

2.3 Selection of Rhizobacteria and Inocula Preparation

Pre-isolated and identified strains of rhizobacteria containing ACC-deaminase obtained from Soil Microbiology and Environmental Sciences Lab, PMAS-AAU, Rawalpindi were used for field trials. Two distinct strains of chickpea Ochrobactrum cicero Ca-34T (DSM 22292; CCUG 57879) and Mesorhizobium cicero TAL-1148 (USDA 3100) were used for inoculation. O. cicero Ca-34T is an IAA producing strain and naturally resistance to ampicillin (10 µg), aztreonam (30 µg), cephradine (ß-lactams 30 µg), cefixime (5 µg), amikacin (30 µg), carbenicillin (100 µg), gentamicin (10 µg), cephradine (30 µg), ceftriaxone (30 µg), paratam (105 µg), kanamycin (30 µg), rifampicin (5 µg), trimethoprim (1.25 µg)/sulfamethoxazole (23.76 µg) (25 µg), and chloramphenicol (30 µg). M. cicero TAL-1148 (provided by the Nitrogen Fixation by Tropical Agricultural Legumes Project, University of Hawaii, Paia, Hawaii, USA) is a reference nodulating strain being used for inoculum production worldwide. O. cicero Ca-34 T and M. cicero TAL-1148 were grown in Luria-Bertani (LB) and Yeast extract-Manitol (YEM) broth, respectively, at 28 ±2°C over-night with constant shaking. The cells were harvested with centrifugation and suspended in saline to get 10 9 cells ml^{-1}. Bacterial cultures were mixed with sterilized finely ground carrier material (finally grinded filter mud). The seeds were mixed until they become coated with a thin film of bacterial inoculum. Seeds for the un-inoculated control were coated in a similar manner with the sterilized carrier material prepared in saline. Coated seeds were air-dried in shade before sowing.

2.4 Soil Sampling and Analysis

Soil samples from study area were analyzed prior to experimentation (Table 1). Soil samples were obtained from 0-15 cm prior to sowing of chickpea from each treatment for soil moisture determination and for physico-chemical estimations. Soil samples were air-dried and passed through 2 mm sieve before chemical analysis. Soil pH and EC_e were calculated by the methods described by [15] and [16] respectively. Soil organic matter by [17], Soil moisture contents were calculated by gravimetric method [18]. All laboratory determinations were carried out at SAWCRS, Fateh Jang.

Table 1. Physico-chemical analysis of selected site before cropping

Parameter	Value	Unit
pH	8.12	-
Saturation	25.40	%
O.M	0.80	%
EC_e	1.74	dS m^{-1}

2.5 Statistical Analysis

The data collected was analyzed statistically by using computer software M-STAT-C. Least significant difference (LSD) was employed to separate the treatment means (P<0.05).

3. RESULTS AND DISCUSSION

Considering the significance of PGPR, different combinations of treatments were tested in field trial on chickpea to further confirm the validity of PGPR in the presence or absence of PGPR. Impact of these treatments on different growth parameters of chickpea grown in field conditions is evident from the data as summarized under:

3.1 Crop Yield Data

At harvest in the 2010-11, 2011-12, 2012-13 and 2013-14 *Rabi* (winter) season, the plants from three 1 m^2 subplots per field were combined; the grain was removed from the straw, then the straw and grain weights were recorded.

3.2 1000-Grain Weight (g)

1000 grain weight varied significantly by treatments over control (Fig. 1). It was observed and recorded that the mean maximum 1000 grain weight was (307g) recorded in T_5 (PGPR+FYM), while the mean minimum 1000 grain weight was (247g) recorded for T_1 (Control). At second position was T_4 (FYM) while the T_3 (PGPR) was at third place. The trend remained the same during all study tenure and it was very evident that PGPR showed its maximum response in presence of FYM which indicates enhanced nutrient use efficiency by application of PGPR. PGPR inoculation caused a maximum increase in 1000-grain weight of 11.7% over control. But PGPR along with FYM yielded highest increase of 30.3% in1000-grain weight, followed by 28.1% increase with FYM over control. Other treatments also showed significant increase in1000-grainweight up to 9.85% compared to control. Several studies were in support of the findings and reported that higher number of grain rows could be due to presence of favourable conditions for growth, developed root systems and more nutrients uptake [19]. [20] also reported that organic/inorganic fertilizers application in maize improved 1000-grain weight.

3.3 Biomass Yield

Biomass yield was significantly affected by amendments including PGPR. Data elaborated that the mean maximum biomass yield was (1862 Kg ha^{-1}) observed in T_5 (PGPR+FYM). PGPR alone was ranked third with (1705 Kg ha^{-1}) biomass yield while T_4 (FYM) produced (1747 Kg ha^{-1}) second mean maximum biomass yield. The mean minimum biomass yield was (1442 Kg ha^{-1}) recorded in control (untreated). The trend of biomass yield increase over control was 12, 15, 17 and 23% by gypsum, PGPR, FYM and PGPR+FYM, respectively. The mechanism of increase in biomass yield may be attributed to various reasons, such as nitrogen fixation, synthesis of phytohormones, production of metabolites, insoluble phosphate solubulization by rhizobacteria [21,22], addition of soil nutrients and organic matter, enhanced water retention and soil structure stability by FYM [23] and water infiltration, soil structure stability and nutrient retention by gypsum [24]. Application of organic material along with PGPR has further enhanced the effectiveness of the PGPR because one of the important mechanisms for these beneficial effects is PGPR-elicited enhanced nutrient availability and nutrient use efficiency [12].

Another important trait of PGPR, that may indirectly influence the plant growth, is the production of ammonia [25]. Results are in conformation with the findings of [26] reported that growth parameters, yield attributes of crops increased significantly with increasing N rate in combination with PGPR + organic manure. These findings may be due to the increased synthesis of hormones like gibberellins, which would have triggered the activity of specific enzymes that promoted early germination, such as a-amylase, which have brought an increase in availability of starch assimilation. Beside, significant increase in seedling vigor would have occurred by better synthesis of auxins [27]. This may also 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 [28,29].

3.4 Grain Yield

Separate use of PGPR improved chickpea growth but the effect of PGPR was more prominent regarding plant growth promotion when it was applied in combination with FYM. However, grain yield was also enhanced significantly with other applied amendments as well. Results of the study shown in the (Fig. 2) indicated that all treatments applied significantly ($p<0.05$) improved the chickpea grain yield compared to control. Data obtained revealed that the mean maximum grain yield (1519 Kg ha^{-1}) was achieved by T_5 (PGPR+FYM) followed by 1415 Kg ha^{-1} by T_4 (FYM) while the mean minimum grain yield (1231 Kg ha^{-}1) was obtained in T_1 (Control). Over all, trend of grain yield increase over control was 6, 11, 13 and 19% by gypsum, PGPR, FYM and PGPR+FYM, respectively.

It was observed that PGPR containing ACC-deaminase along with FYM showed highly positive effect on growth and yield of chickpea as compared to control. Organic materials not only serve as a source of plant macro- and micro-nutrients but also support the activity of inocula [30,31,32]. So application of FYM affected the growth of chickpea by enhancing nutrient mobilization and uptake, in addition to enhancing bacterial activities. PGPR are known to affect the nutrient availability to the plant through mineralization, acidification, redox changes or by producing iron cheaters and siderophores [33, 34]. Similarly PGPR have been shown to solubilize precipitated phosphates and enhance phosphate availability to chickpea that represent a possible mechanism of plant growth promotion under field condition [35]. This is different from the observations of[36], who reported no significant difference in grain and biomass yield of chickpea, respectively, when inoculant alone or fertilizer alone was used while in this study PGPR alone ranked third. However, results presented here support the hypothesis that organic basal fertilizer with PGPR alone or in combinations and soil additives can improve plant growth and the nutrient contents part of the plants [37]. On the other hand, many studies reported that the application of manure increased the forage and grain yield in many crops [38, 39]. It was further confirmed by [40] who stated that

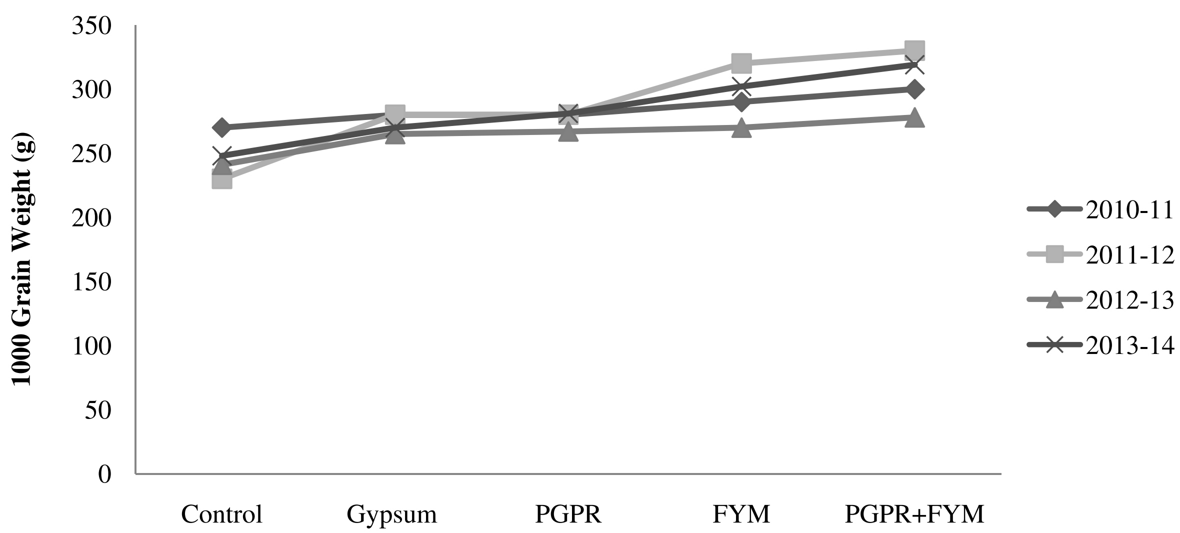

Fig. 1. Effect of amendments on 1000 grain yield

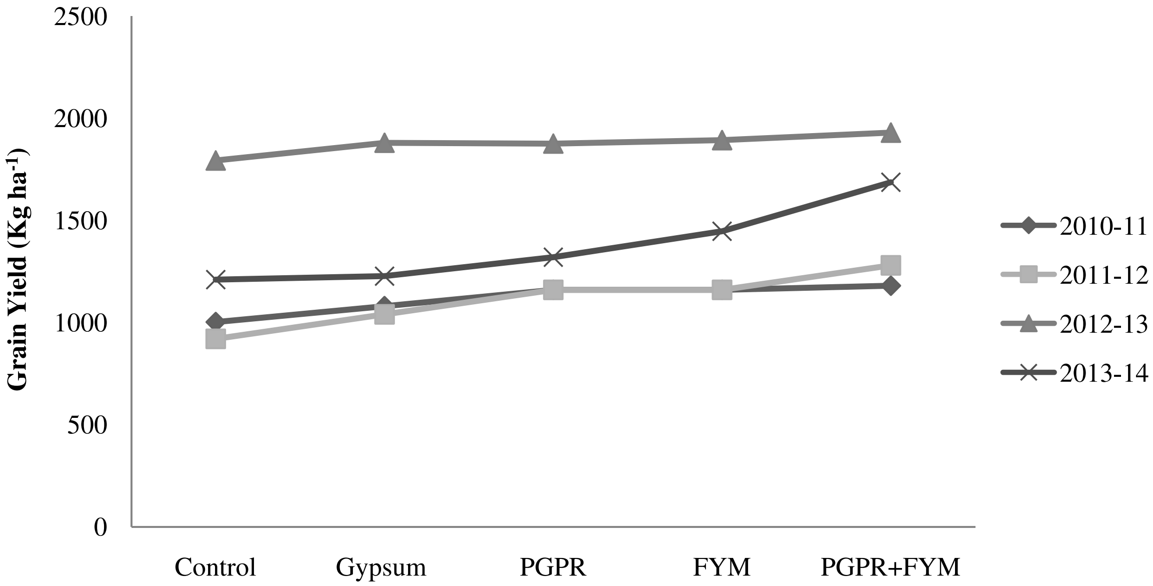

Fig. 2. Effect of amendments on grain yield

integrated use of PGPR containing ACC-deaminase with nitrogenous fertilizer increased the number of nodules, fresh and dry weight of nodules significantly as compared to PGPR and/or nitrogenous fertilizer alone, most likely by decreased C_2H_2 levels in the plant roots during early stages of development and concluded that PGPR containing ACC-deaminase could be used to increase nodulation in legumes.

3.5 Variation of Soil Properties

Bacterial applications changed soil properties significantly (Table 2). At the beginning of this study, soil pH was alkaline (8.12). However, after applications amendments total pH ranged between, 7.81-8.10. The lowest value was in PGPR+FYM and the highest was in control. This result may be explained that PGPR decrease pH due to their organic acid production as a secondary metabolite which can a better soil condition for crop growing [41]. Such decrease in pH values could be attributed to the production of CO_2 and organic acids by soil microorganisms, acting on the soil organic matter. The trend was in agreement with [42]. Soil electrical conductivity is one of soil important character. Data in (Table 2) showed a significant effect on soil electrical conductivity as affected by all soil treatments with different amendments. The EC varied from 1.73 to 1.30 dSm^{-1}. This result may be attributed to the combination effect PGPR, FYM and gypsum, caused the decrease salt in the soil by retaining soil moisture. Similar results were found by [43].

Table 2. Monthly rainfall (mm) during experimental period in Fateh Jang (2010-14)

Month	2010-11	2011-12	2012-13	2013-14
Oct	54	23	36	59
Nov	0	27	0	15
Dec	9	0	89	3
Jan	7	45	4	3
Feb	66	33	351	67
March	45	8	68	137
April	62	137	70	35
Total	243	273	618	319

Source: SAWCRS, Fateh Jang

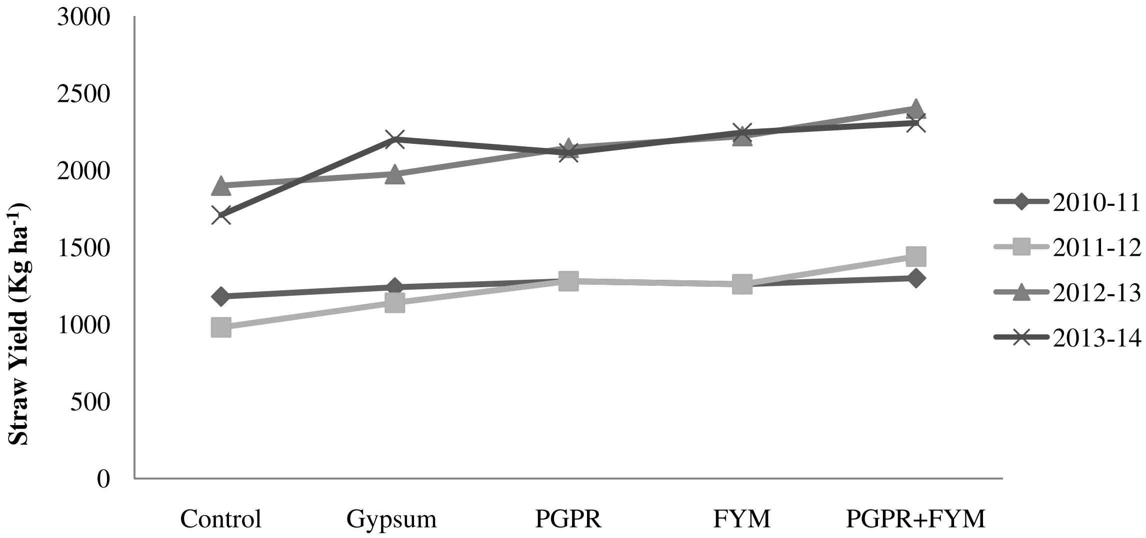

Fig. 3. Effect of Amendments on straw yield

Table 3. Chemical properties of the studied soil under different treatments after chickpea harvest

Treatment	Year	Saturation (%)	pH	EC (d Sm^{-1})	Organic matter (%)
Control	2010-11	25.52	8.10	1.73	0.78
	2011-12	25.5	8.10	1.70	0.74
	2012-13	25.58	8.09	1.71	0.74
	2013-14	25.50	8.07	1.70	0.72
	Mean	25.52	8.09	1.71	0.74
Gypsum	2010-11	25.66	8.10	1.76	0.78
	2011-12	25.76	8.08	1.77	0.79
	2012-13	26.16	8.06	1.79	0.81
	2013-14	26.10	8.04	1.89	0.80
	Mean	25.92	8.07	1.80	0.80
PGPR	2010-11	25.48	8.09	1.72	0.80
	2011-12	25.68	8.09	1.71	0.84
	2012-13	25.88	8.03	1.67	0.89
	2013-14	25.58	8.00	1.66	0.92
	Mean	25.66	8.04	1.69	0.86
FYM	2010-11	25.8	8.02	1.70	0.84
	2011-12	26.02	8.01	1.66	0.88
	2012-13	27.00	7.98	1.60	0.95
	2013-14	27.10	7.90	1.51	1.01
	Mean	26.48	7.98	1.62	0.92
FYM+PGPR	2010-11	26.01	8.00	1.60	0.92
	2011-12	26.21	7.96	1.42	0.96
	2012-13	27.21	7.90	1.37	1.02
	2013-14	28.20	7.81	1.30	1.16
	Mean	26.91	7.92	1.42	1.02

The levels of organic matter showing a moderate increase in treatments with gypsum and PGPR applied alone but FYM and its combination with PGPR has shown better response (Table 2). However, the lowest organic matter was in control and increased by 8, 14, 20 and 27 % through gypsum, PGPR, FYM and FYM+PGPR, respectively. This may be due to the plant growth promoting characteristics of PGPR and increasing organic matter mineralization through the process of acidification, chelation and exchange reactions which improved 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 [44, 45]. Application of soil amendments enhanced the saturation % of studied soil. It was revealed that the lowest soil saturation (25.52%) was in control while the highest soil saturation (26.91%) was recorded in PGPR+FYM treatment. The trend of increase in soil saturation was 2, 1, 4 and 5% by gypsum, PGPR, FYM and PGPR+FYM, respectively. This could be due to improved soil conditions and reduction in soil bulk density [46].

4. CONCLUSION

The study revealed that PGPR application has increased the fertilizer use efficiency. In view of environmental pollution in case of excessive use of mineral fertilizers and due to high costs in the production of N and P fertilizers, bacteria tested in the study may well be suited alone or in combination to achieve sustainable and ecological agricultural production in the region. An important nutritional problem of developing countries is diminishing soil fertility, also called hidden hunger. This paper supports the view that inoculations with PGPR have some potential to increase use efficiency of organic fertilizer in both organic and conventional farming.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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