

Original Research Article

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## Enhancing the Nutrient Uptake and Quality of Pearlmillet (*Pennisetum glaucum* L.) through Use of Biofertilizers

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### ABSTRACT

Inoculation of biofertilizers leads to reduction of chemical fertilizer requirement by fixation & solubilisation of plant nutrients and thus, may able to maximize plant growth, yields and quality of output. To study the beneficial effect of biofertilizers on performance of pearlmillet (*Pennisetum glaucum* L.) a field experiment was conducted in randomized block design during the rainy (*Khari*) season of 2014 at Sam Higginbottom University of Agriculture, Technology and Sciences, Allahabad, India. Seeds of pearlmillet were treated with diazotrophic bacteria namely- *Pseudomonas fluorescens*, *Azotobacter chroococcum*, *Azospirillum lipoferum*, *Acetobacter diazotrophicus* and one fungi- *Trichoderma viride* @ 10-25 g kg<sup>-1</sup> alone and in combinations. The combined treatment with all the bioinoculants enhanced the grain yield (44%), nutrient uptake (N by 79.9% and P by 87.9%) and grain quality (Protein by 58.9% and carbohydrate by 17%), single inoculation was also found profitable over the control (Un-inoculated). Therefore, inoculation of pearlmillet seed with different biofertilizers could produce pollution free and healthy (better quality) food for increasing population and may able to reduce chemical fertilizer application without any significant reduction in grain yield.

#### Keywords

*Pseudomonas fluorescens*,  
*Azotobacter chroococcum*,  
*Azospirillum lipoferum*,  
*Acetobacter diazotrophicus*,  
*Trichoderma viride* and Pearl  
millet

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### Introduction

Pearlmillet [*Pennisetum glaucum* (L.)] is a drought tolerant, warm season cereal crop, largely grown for grain and fodder purpose and ranking sixth in the world following rice, wheat, corn, barley and sorghum. Pearlmillet can be grown in areas which are very hot, dry and soils having very poor organic matter, low water holding capacity and high infiltration rate. It can also grow in areas receiving less than 400 mm annual precipitation, which limit

the growth of other cereal crop, due to its well-developed deep root system, short life cycle and fast growing habit. India is the major pearlmillet producing country in the world with Rajasthan sharing the major production (46%) followed by Maharashtra (19%), Gujarat (11%), Uttar Pradesh (8%) and Haryana (6%) (Sonawane *et al.*, 2010). Pearlmillet grain contains about 13-14 per cent protein, 5-6 per cent fat, 74 per cent carbohydrate, 1-2 per cent minerals and higher amount of carotene, riboflavin (Vitamin B<sub>2</sub>)

and niacin (Vitamin B<sub>4</sub>) (Singh *et al.*, 2009). High yielding potential along with nutritional features make pearl millet an important cereal crop that can effectively address the emerging challenges of food and nutritional security, global warming, water shortages and land degradation.

Pearlmillet crop is generally grown in marginal and sub-marginal lands (Parihar *et al.*, 2010). Farmers often targeting higher yields tend towards higher use of fertilizers which often remains under-utilized in soils. Moreover, chemical fertilizers used to supply nutrients results, more cost of cultivation and severe environmental pollution (Dai *et al.*, 2004) and also deteriorate the grain quality (Scheiner *et al.*, 2002). Previous research provides ample evidence that a part of those harmful fertilizers can be replaced by biofertilizers (El Kholly *et al.*, 2005). Compared to chemical fertilizers, biofertilizers are cost effective, renewable and eco-friendly sources of nutrient supply. Nitrogen fixer (Mrkovacki and Milic, 2001; Chen, 2006), phosphate and other nutrient solubilizing microorganisms (Cattelan *et al.*, 1999) play an important role in supplementing nutrient to the plant, allowing a sustainable use of nitrogen and phosphate fertilizers (Tambekar *et al.*, 2009). Certain strains are referred to as plant growth promoting rhizobacteria (PGPR), which can be used as inoculant biofertilizers (Kennedy *et al.*, 2004) are: *Pseudomonas*, *Bacillus*, *Azospirillum*, *Azotobacter*, *Burkholderia*, *Rhizobium*, *Erwinia*, *Mycobacterium* and *Flavobacterium*. Some species of *Azotobacter* and *Azospirillum* bacteria are able to provide beneficial effect on plant growth and pest resistance (Lugtenberg *et al.*, 2002) directly and/or indirectly (Persello-Cartieaux *et al.*, 2003; Nelson, 2004). Biofertilizers colonizes the rhizosphere and promotes growth and yield (Salantur *et al.*, 2006) by enhancing the germination (Raju *et al.*, 1999; Niranjan *et al.*,

2003; Niranjan *et al.*, 2004) availability and supply of nutrients, along with production of antibiotic (Zahir *et al.*, 2004) and phytohormones (Egamberdiyeva, 2007) to crop when the seed is treated or with soil application. Pearl millet and sorghum crops inoculated with *Azotobacter* and *Azospirillum* recorded 11-12% increased yields in dryland condition (Wani, 1990). *Acetobacter sp.* is a new nitrogen fixing bacterium, isolated from sugarcane (Sharma, 2012) which act as a biofertilizers and enhance the performance of different crops with less fertilizer use (Jhala *et al.*, 2014). Hungria *et al.*, (2010) and Mellado *et al.*, (1998) reported *Azospirillum* and *Acetobacter* as best endophytic N fixers in maize crop. *Trichoderma sp.* is also helpful for enhancing the growth, productivity and health (Harman, 2006) by increasing the accessibility of plant nutrients, minerals (Fe, N, P), production of growth hormones (Kaewchai *et al.*, 2009), providing protection against drought stress (Mastouri *et al.*, 2010), mycoparasitism, production of antibiotics and alter plant gene expression for inducing resistance in plants against several diseases (Alfano *et al.*, 2007; Pandya and Saraf, 2010).

Inoculation of nitrogen fixing and phosphate solubilising microorganisms alone or in combination increase plant height, number of tillers and ultimately the yield (Saxena, 1997) and reduce the use of chemical fertilizers to supply nutrient requirement of the pearl millet (Aggarwal and Venkateswarlu 1989). El-Kholly and Goma (2000) and Urquiaga *et al.*, (1992) have succeeded in reducing the recommended doses of chemical fertilizers needed for corn and millet by 50 % using biofertilizers without any loss in yield. There are studies elucidating the effect of biofertilizers alone or in combination with other chemical fertilizers (Ram Rao *et al.*, 2007; Seema *et al.*, 2000). However, a systematic study involving both bacterial and fungal strains as inoculants is very meagre.

The aim of the present study was to explore the possibilities of which single biofertilizers or combination of different biofertilizers gave the best results in terms of yield, nutrient uptake and quality of pearl millet crop.

## Materials and Methods

### Experimental site

A field experiment was carried out at Crop Research Farm, Department of Agronomy, Allahabad School of Agriculture, SHUATS, Allahabad (U.P.), located at 25°24' 42" N latitude, 81° 50' 56" E longitude and 98 m altitude above the mean sea level, during the rainy (*Kharif*) season of 2014 to assess the effects of biofertilizers on performance of pearl millet crop (*Pennisetum glaucum* L.). The soil was sandy loam in texture, with pH 7.4, organic carbon 0.32%, low available N 188.3 kg ha<sup>-1</sup>, medium available P 34.5 kg ha<sup>-1</sup>, available K 130.5 kg ha<sup>-1</sup>. The total rainfall received during the crop season was 48.86 mm.

### Experimental treatments

Pearl millet variety, UMA 7111 (duration- 85-90 days) was treated with the diazotrophic bacteria namely- *Pseudomonas fluorescens*, *Azotobacter chroococcum*, *Azospirillum lipoferum*, *Acetobacter diazotrophicus*, and one fungi- *Trichoderma viride* alone and in combinations @ 10-25 g kg<sup>-1</sup>, and sown in randomized block design with three replications. The net plot area was 9 m<sup>2</sup> and inoculated seeds were sown (below 1 cm) at row spacing of 45 cm. while, plant to plant spacing was 15 cm. Nitrogen and phosphorus were applied uniformly to each treatment including control @ 50 and 30 kg ha<sup>-1</sup> (50 percent of RDF) through urea and diammonium phosphate respectively. Whole amount of nitrogen and phosphorus was applied at 15 DAS because it affects the

establishment and multiplication of biofertilizers, if applied at the time of sowing. To assess the effects of biofertilizers, different treatments were worked at after harvest. The grain yields were recorded on plot basis and then converted in to q ha<sup>-1</sup>. For nutrient content determination, nitrogen content was determined by Kjeldahl method using alkaline permanganate (Subbiah and Asija, 1956) and phosphorus levels by Olsen's method, 1954, protein content was determined by Lowry *et al.*, 1951 method and carbohydrate by Colorimetric method. The nutrient uptake by the crops was obtained as the product of concentration of nutrient and yield. Analysis of variance (ANOVA) was done as per the procedure outlined by Gomez and Gomez (1984). The significant differences between treatments were compared with the least significance (LSD) at 5% level of probability.

## Results and Discussion

### Influence of biofertilizers on nutrient content and uptake

In the present study it was observed that combination of different biofertilizers was able to increase N and P content in grain and stover significantly (Table 1). The maximum N content (1.96% in grain & 0.74% in stover) and P content was found (0.306% in grain & 0.081% in stover) with treatment, *P. fluorescens* + *A. chroococcum* + *A. lipoferum* + *A. diazotrophicus* + *T. Viride*. Nitrogen and phosphorus uptake in grain and stover was also significantly increased due to combined use of biofertilizers. The maximum N uptake (59.03 kg ha<sup>-1</sup> in grain and 79.76 kg ha<sup>-1</sup> in stover) and P uptake was found (9.21 kg ha<sup>-1</sup> in grain and 8.71 kg ha<sup>-1</sup> in stover) (Table 2) with treatment *P. fluorescens* + *A. chroococcum* + *A. lipoferum* + *A. diazotrophicus* + *T. Viride*. Increased N and P uptake was recorded with biofertilizers combinations mainly due to systematic

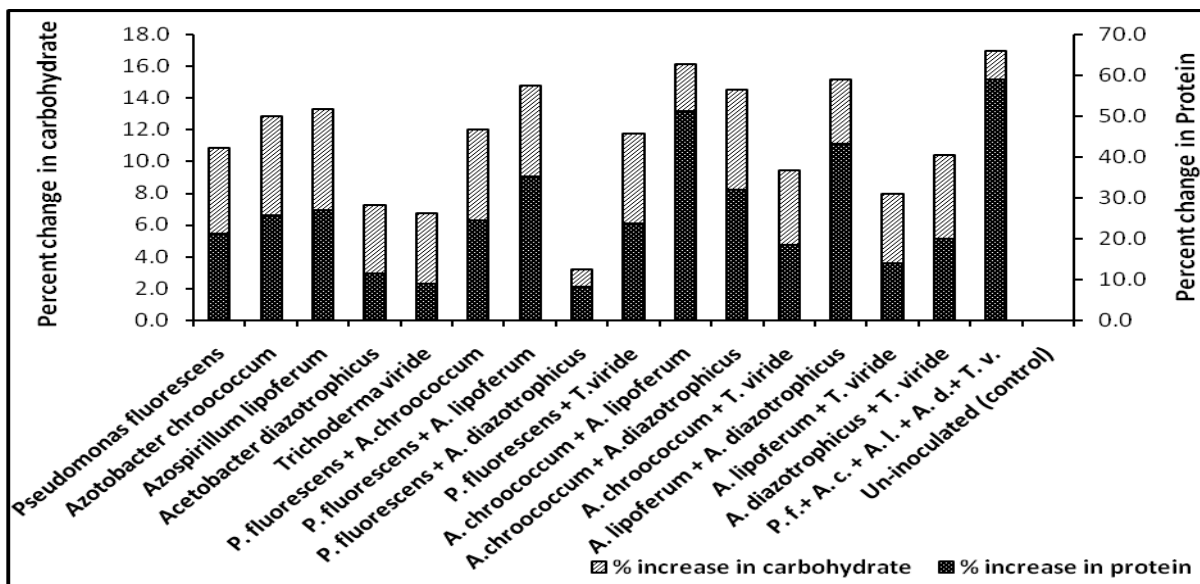
activity of biofertilizers to enhance the availability of nutrients (Belimov *et al.*, 1995) by fixation of nitrogen and converting the fixed phosphorus in soil to plant available form (Richardson, 2001) and production of plant growth-regulating hormones (Sahin *et al.*, 2004) and enzymes resulting in improved plant root morphology and physiology (Lugtenberg and Dekkers, 1999). Total N and P uptake (grain + stover) was to the tune of 138.79 and 17.95 kg ha<sup>-1</sup> respectively, with the combined application of all the biofertilizers together, which was 79% and 87.96% (total N and P) higher over un-inoculated treatment. This view was consistent with the results of Bashan *et al.*, (2004), Van der Heijden *et al.*, (2006), Farzaneh *et al.*, (2011) with the application of single or combined effect of biofertilizers.

**Influence of biofertilizers on grain and stover yield**

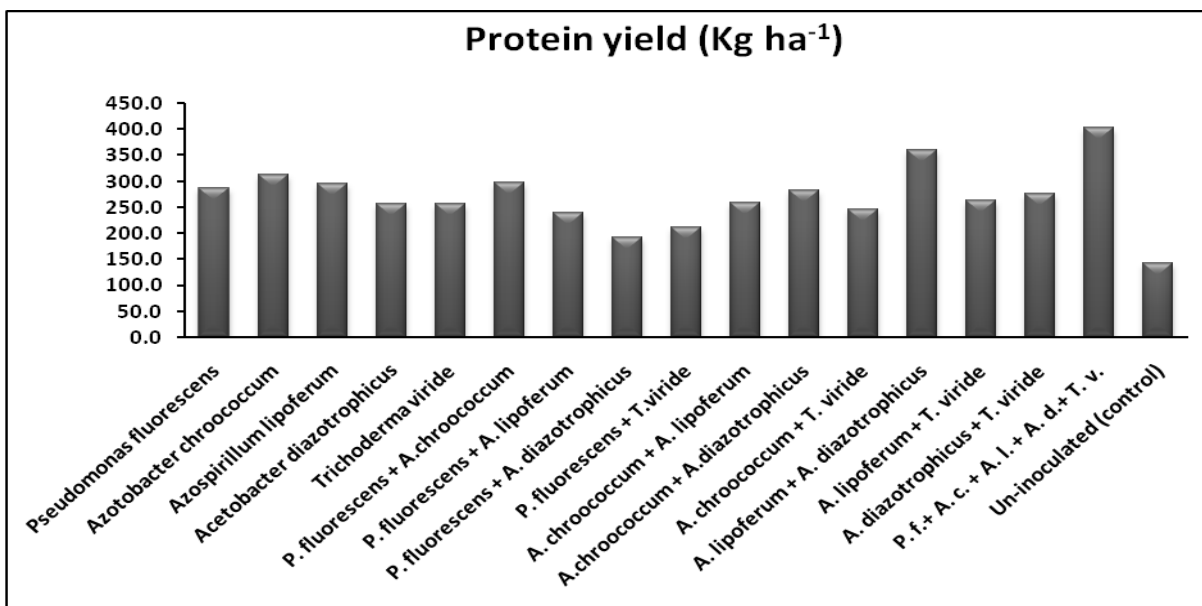
Yield (grain and straw) of pearl millet exhibited significant variation due to use of biofertilizers (Table 2). The highest grain yield (30.1 q ha<sup>-1</sup>) and straw yield (107.7 q

ha<sup>-1</sup>) was recorded in treatment (*P. fluorescens* + *A. chroococcum* + *A. lipoferum* + *A. diazotrophicus* + *T. Viride*) followed by (*A. lipoferum* + *A. diazotrophicus*) and (*Azotobacter chroococcum*), while lowest yield was observed in control (Un-inoculated). Due to use of biofertilizer combinations grain and stover yield was increased by 79 percent 23 percent respectively over the control. The yield was significantly influenced with the application of biofertilizers (Belimov *et al.*, 1995; Cakmakci *et al.*, 2001) which may be attributed to the increased uptake of nutrients by the plants, improvement of soil characteristics such as contents of organic materials and increase in available nutrient to the crop. Nutrient cycling, decomposition of organic matter, promoting plant growth and health are the major activities of biofertilizers. It was also observed that biofertilizers combined with fertilizer application helped in Nitrogen fixation, phosphate solubilization, production of hormones and antibiotics which were responsible for increased plant growth and yields (Pal, 1999; Asghar *et al.*, 2002 and Ozturk *et al.*, 2003).

**Fig.1** Percent change in carbohydrate and protein content of pearl millet over the control as influenced by the inoculation of biofertilizers



**Fig.2** Protein yield (Kg ha<sup>-1</sup>) of pearl millet as influenced by the inoculation of biofertilizers



**Table.1** Nitrogen and phosphorus content (%) of Pearlmillet as influenced by the inoculation of biofertilizers

Treatments	Nitrogen content (%)		Phosphorus content (%)	
	Grain	Stover	Grain	Stover
<i>Pseudomonas fluorescens</i>	1.69	0.63	0.300	0.076
<i>Azotobacterchroococcum</i>	1.82	0.71	0.282	0.067
<i>Azospirillumlipoferum</i>	1.83	0.71	0.280	0.066
<i>Acetobacter diazotrophicus</i>	1.70	0.69	0.277	0.063
<i>Trichoderma viride</i>	1.68	0.64	0.272	0.061
<i>P.fluorescens</i> + <i>A.chroococcum</i>	1.80	0.70	0.302	0.078
<i>P.fluorescens</i> + <i>A.lipoferum</i>	1.81	0.70	0.303	0.080
<i>P.fluorescens</i> + <i>A. diazotrophicus</i>	1.67	0.66	0.301	0.077
<i>P.fluorescens</i> + <i>T.viride</i>	1.79	0.70	0.298	0.075
<i>A.chroococcum</i> + <i>A.lipoferum</i>	1.93	0.73	0.296	0.074
<i>A.chroococcum</i> + <i>A.diazotrophicus</i>	1.86	0.72	0.290	0.072
<i>A.chroococcum</i> + <i>T. viride</i>	1.72	0.68	0.288	0.069
<i>A.lipoferum</i> + <i>A. diazotrophicus</i>	1.91	0.72	0.292	0.073
<i>A.lipoferum</i> + <i>T. viride</i>	1.69	0.67	0.284	0.068
<i>A. diazotrophicus</i> + <i>T. viride</i>	1.72	0.68	0.290	0.071
<i>P.f.</i> + <i>A.c.</i> + <i>A. l.</i> + <i>A. d.</i> + <i>T. v.</i>	1.96	0.74	0.306	0.081
Un-inoculated (control)	1.54	0.59	0.261	0.059
CD (p= 0.05)	<b>0.211</b>	<b>0.078</b>	<b>0.010</b>	<b>0.009</b>

*P. f.* - *Pseudomonas fluorescens* (10 g kg<sup>-1</sup> of seed), *A. c.* - *Azotobacter chroococcum* (25 g kg<sup>-1</sup> of seed), *A. l.* - *Azospirillum lipoferum* (10 ml kg<sup>-1</sup> of seed), *A.d.* - *Acetobacter diazotrophicus* (20 g kg<sup>-1</sup> of seed) and *T. v.* - *Trichoderma viride* (10 g kg<sup>-1</sup> of seed).

**Table.2** Grain yield, straw yield and uptake of nitrogen and phosphorus of pearl millet as influenced by the inoculation of biofertilizers

Treatments	Grain Yield (q ha <sup>-1</sup> )	Stover Yield (q ha <sup>-1</sup> )	Nitrogen uptake (Kg ha <sup>-1</sup> )		Total Nitrogen uptake (Kg ha <sup>-1</sup> )	Phosphorus uptake (Kg ha <sup>-1</sup> )		Total Phosphorus uptake (Kg ha <sup>-1</sup> )
			Grain	Stover		Grain	Stover	
<i>Pseudomonas fluorescens</i>	27.6	92.9	47.24	58.73	105.98	8.41	7.03	15.44
<i>Azotobacter chroococcum</i>	29.4	101.0	53.64	71.60	125.24	8.29	6.82	15.11
<i>Azospirillum lipoferum</i>	27.4	82.2	50.11	58.56	108.67	7.68	5.43	13.11
<i>Acetobacter diazotrophicus</i>	27.3	81.4	46.62	55.94	102.56	7.53	5.15	12.67
<i>Trichoderma viride</i>	27.8	92.7	46.77	59.26	106.04	7.56	5.64	13.20
<i>P. fluorescens</i> + <i>A. chroococcum</i>	28.2	98.3	50.63	69.14	119.77	8.54	7.66	16.19
<i>P. fluorescens</i> + <i>A. lipoferum</i>	20.8	77.6	37.66	54.02	91.68	6.32	6.23	12.55
<i>P. fluorescens</i> + <i>A. diazotrophicus</i>	21.0	84.4	35.39	55.21	90.59	6.32	6.50	12.83
<i>P. fluorescens</i> + <i>T. viride</i>	20.1	83.2	36.08	58.30	94.38	6.01	6.20	12.21
<i>A. chroococcum</i> + <i>A. lipoferum</i>	20.2	79.6	39.11	58.36	97.47	5.97	5.87	11.84
<i>A. chroococcum</i> + <i>A. diazotrophicus</i>	25.4	89.2	47.36	63.99	111.36	7.37	6.35	13.72
<i>A. chroococcum</i> + <i>T. viride</i>	24.5	88.8	42.39	60.91	103.30	7.08	6.15	13.23
<i>A. lipoferum</i> + <i>A. diazotrophicus</i>	29.7	102.4	56.69	73.49	130.18	8.69	7.41	16.11
<i>A. lipoferum</i> + <i>T. viride</i>	27.4	78.4	46.19	52.32	98.51	7.76	5.30	13.06
<i>A. diazotrophicus</i> + <i>T. viride</i>	27.2	88.3	46.71	59.66	106.38	7.89	6.22	14.12
<i>P.f.</i> + <i>A.c.</i> + <i>A. l.</i> + <i>A. d.</i> + <i>T. v.</i>	30.1	107.7	59.03	79.76	138.79	9.21	8.73	17.95
Un-inoculated (control)	16.8	87.4	25.93	51.23	77.15	4.39	5.16	9.55
CD (p= 0.05)	<b>3.9</b>	<b>19.6</b>	<b>8.58</b>	<b>15.84</b>	-	<b>1.01</b>	<b>1.49</b>	-

## **Influence of biofertilizers on quality of grain**

Effect of combined application of biofertilizers was found significant on protein content, but carbohydrate content was not affected significantly. The maximum protein content (13.43%) and carbohydrate content 68.7 g/100g (Fig. 1) was recorded with the treatment *P. fluorescens* + *A. chroococcum* + *A. lipoferum* + *A. diazotrophicus* + *T. Viride*. Compared to un-inoculated treatment, protein and carbohydrate content was increased by 58.9 % and 17% respectively (Fig. 1).

Protein yield of pearl millet crop was mainly increased because it was directly related to the yield and protein content of the crop. The maximum protein yield 404.33 kg ha<sup>-1</sup> (Fig. 2) was found with treatment *P. fluorescens* + *A. chroococcum* + *A. lipoferum* + *A. diazotrophicus* + *T. Viride*.

Previous researchers have proved that biofertilizers are cheapest and most effective tool for obtaining sustainable yields in agriculture (Dakora, 2003), some free-living bacteria were found to have not only the ability to fix nutrients but also the ability to release phytohormones (Sahin *et al.*, 2004) similar to gibberellic acid (Joo *et al.*, 2004) and indole acetic acid, which could stimulate plant growth, absorption of nutrients, and quality of crop. Inoculation with PSB, improved solubilization of fixed soil P and applied phosphates, resulting in more availability of phosphorus to the plants (Whitelaw *et al.*, 1997; Whitelaw, 2000).

An improvement of yield and quality could be attributed to the high nitrogen uptake by the biofertilizers inoculated plants as well as the ability of the organisms to produce growth promoting substances (Kim *et al.*, 1997). In general, the effect of inoculants was observed to be superior over the control. Growth

substances produced by microorganisms might enhance the plant growth, resulting in higher canopy development of plants leading to better utilization of sun rays to convert it into food materials and better root development of plant help to enhance water and nutrient uptake and also provide more strength to plant by increasing the area of rhizosphere.

So, on the basis of above findings it may be concluded that combined inoculation of all biofertilizers might help in increasing the crop yield with better quality (Fukui *et al.*, 1994). The present findings were also corroborated by Bhagchand and Gautam, (2000) who reported that the use of biofertilizer lead to higher availability of nitrogen and phosphorus that promoted growth and development of the crop (Latake *et al.*, 2009) and ultimately resulting in higher yields.

The use of biofertilizers can also substitute a part of chemical fertilizers (Latake *et al.*, 2009) resulting in reducing the pressure of increasing population on the soil and environmental pollution (Karmakar *et al.*, 2007) and also enhancing the sustainability of the system concurrently (Bloemberg *et al.*, 2000).

Biofertilizers are inexpensive and ecologically sound source of plant nutrients. Pearl millet crop due its adverse growing conditions under restricted moisture regimes, often leave untapped nutrients in the soil. So, for reducing the need for chemical fertilizers in agriculture and improving the nutrient use efficiency, it is important to increase and extend the role of biofertilizers for decreasing adverse environmental effects.

On the basis of present investigation and above results it may be concluded that combinations of diazotrophic bacteria *Pseudomonas fluorescens*, *Azotobacter*

*chroococcum*, *Azospirillum lipoferum* and *Acetobacter diazotrophicus* along with *Trichoderma viride* significantly increased the yield, nutrient content, uptake and quality of pearl millet crop. Even use of single or combination of two or more biofertilizers might be much advantageous for farmers.

Biofertilizers may help in reducing the soil, water and air pollution by substituting some percentage of chemical fertilizers. Therefore, widespread adoption of biofertilizers may provide a viable solution to the farmers, in securing yield and crop quality without dislocating the natural resources (soil, air and water) and contribute to doubling farmer income and simultaneously attempt to meet the national goal.

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