



Original Research Article

Effect of Combined Inoculations of Plant Growth Promoting Rhizobacteria (PGPR) on the Growth and yield of groundnut (*Arachis hypogaea* L.)

S.Mathivanan*, AL.A.Chidambaram, P. Sundramoorthy,
L.Baskaran and R.Kalaikandhan

Department of Botany, Annamalai University, Annamalai nagar, Tamilnadu, India

*Corresponding author

ABSTRACT

Keywords

Plant growth promoting rhizobacteria, groundnut, growth, yield, *Rhizobium*, *Pseudomonas* and *Bacillus*

The study was carried out to find out the effect of combined inoculations of Plant Growth Promoting Rhizobacteria (PGPR) on the productivity of Groundnut (*Arachis hypogaea* L.) under pot culture experiments. The seed inoculations of *Rhizobium*, *Pseudomonas* and *Bacillus* were tested in different combinations on the growth and yield components of Groundnut. All the inoculation treatments showed better plant growth, nutrient uptake and yield when compared to uninoculated control. Among various combinations, single inoculations performed better than uninoculated control while dual inoculations found better over single inoculation treatments. Combined inoculations of three beneficial organisms viz., *Rhizobium*, *Pseudomonas* and *Bacillus* were more superior over both single and dual inoculations, however combined inoculation of three organisms with respect to plant growth, yield and nutrient uptake.

Introduction

Groundnut (*Arachis hypogaea* L.) is a premier oil seed crop of India occupying 45 percent of total oil seed production. India ranks first in the world accounting for 30 percent of the world's groundnut area (690 M ha) and 19 percent of world's production (6.41 MT). Though India leads both in area and production of groundnut, the country ranks eight in productivity due to fertilizer management and erratic response of the crop in terms of yield. Intensive groundnut cultivation largely requires use of chemical fertilizer even though they are expensive

besides short supply. The escalating cost of fertilizers and low Purchase power of peasants restrict judicious usage of these inputs under such conditions, there is every need to explore the possibilities of using the native sources of plant nutrition. The bio and organic sources, like biofertilizers and organic manures as supplement to plant nutrients are gaining global importance in groundnut cultivation. Plant Growth Promoting Rhizobacteria (PGPR) is a group of bacteria that enhances plant growth and yield through various growth promoting

substances as biofertilizers. The use of soil microorganisms (PGPR) for sustainable and safe agriculture has increased globally during the last couple of decades. PGPR as biofertilizer is well recognized as efficient soil microbes for sustainable agriculture and hold great promise in the improvement of agricultural yields.

Material methods

Seed Materials

The seeds of groundnut (*Arachis hypogaea* L.) var. VRI 2 were obtained from Regional Research Station of Tamil Nadu Agricultural University, Virudhachalam, Cuddalore District, Tamil Nadu, and India.

Plant Growth Promoting Rhizobacteria

Plant growth promoting rhizobacteria (*Rhizobium*, *Pseudomonas*, and *Bacillus*) were obtained from the Department of Microbiology, Faculty of Agriculture, Annamalai University, Annamalai nagar, Tamil Nadu.

Pot culture Experiment

Pot experiments were conducted in Botanical Garden, Department of Botany, Annamalai University, Annamalainagar, Tamil Nadu, India. The experiments were conducted during March 2012 to June 2012. (The experimental site was situated at 11.24 N latitude and 79.41E longitude with an attitude of 5.79 M above Mean Sea Level (MSL).

Seed Treatment

The seeds of groundnut were surface sterilized with 80 percent ethanol and 0.1 percent mercuric chloride and washed the seeds with sterile distilled water for 3 to 4 times. The seeds were mixed with carrier

based plant growth promoting rhizobacteria, either as individual organisms or consortium of organisms separately having a cell load of 1×10^9 CFU/ ml⁻¹ and shade dried for 30 min. After shade drying, the seeds were sown.

Experimental Details

Treatments	Plant growth promoting rhizobacteria
T ₀	Seed without PGPR
T ₁	<i>Rhizobium</i>
T ₂	<i>Pseudomonas</i>
T ₃	<i>Bacillus</i>
T ₄	<i>Rhizobium</i> + <i>Pseudomonas</i>
T ₅	<i>Rhizobium</i> + <i>Bacillus</i>
T ₆	<i>Pseudomonas</i> + <i>Bacillus</i>
T ₇	<i>Rhizobium</i> + <i>Pseudomonas</i> + <i>Bacillus</i>

Growth parameters

Shoot length and root length (cm/plant)

Five plants were randomly selected for recording the root length and shoot length of crop plants. They were measured by using centimeter scale and recorded.

Nodulation (nodule/plant)

Five plants from each treatment with intact roots were removed with the help of digging fork. The root with root nodules were carefully separated from the soil and washed thoroughly and the number of nodules per plant were counted and recorded.

Total leaf area (Kalra and Dhiman, 1977)

Five plant samples were collected at various sampling days and the length and breadth of the leaf samples were measured and recorded. The total leaf area was calculated by using the Kemp's formula:

$$\text{Total Leaf area} = L \times B \times K$$

Where L = Length, B = Breadth and K = Kemp's constant (for dicot 0.66)

Fresh weight and dry weight (mg/plant)

Five plant samples were randomly selected at regular intervals (25, 50, 75th and 100 DAS). They were separated into root and shoot. Their Fresh weight was taken by using an electrical single pan balance. The fresh plant materials were kept in a hot air oven at 80°C for 24 hrs and then their dry weight were also determined.

Yield parameters

Number of seeds per plant

The seeds were removed from the pod and the number of seeds was counted and they were expressed in number of seeds per plant.

Number pods per plant

The pods were removed from the plant was counted and they were expressed in number of pods per plant.

Hundred seed weight (g/seed)

100 matured seeds were collected from test crop and their dry weight was recorded by using an electrical single pan balance.

Soil analyses

The soil samples were collected from control and treated pots before sowing and after

harvesting and labeled separately. Their physico-chemical properties such as pH, Electrical conductivity, nitrogen, phosphorus, potassium, copper, zinc, iron and manganese were estimated and recorded.

Statistical analysis

Statistically significance was assessed at the P<0.05 level using one way ANOVA and means were separated by Duncan's multiple range test (P<0.05) with the help of SPSS 16 software. Means and \pm standard deviations were calculated from three replicates.

Result and Discussion

Pot culture- experiment-Morphological parameters

Pot culture experiment was conducted to find out the effect of Plant growth promoting rhizobacteria on growth, yield content of groundnut (*Arachis hypogaea* L.). The growth and yield of groundnut crop grown under various treatment of Plant growth promoting rhizobacteria application are shown in Plates 2 and 3.

Shoot length (cm/plant)

The results on the effect of Plant growth promoting rhizobacteria on the shoot length (cm/plant) of groundnut crop at various stages of its growth (25, 50, 75 and 100 DAS) are shown in Table 1. The highest shoot lengths (9.4, 25.7, 38.2 and 47.8 cm/plant) were recorded in groundnut crop grown in *Rhizobium* + *Pseudomonas* + *Bacillus* treatment. The lowest shoot length (5.5, 12.1, 23.1 and 30.7 cm/plant) was recorded at various stages of its growth (25, 50, 75 and 100 DAS) in the crop grown without Plant growth promoting rhizobacteria treatment.

Root length (cm/plant)

The results on the effect of Plant growth promoting rhizobacteria on root length of groundnut at various stages of its growth (25, 50, 75 and 100 DAS) are shown in Table 2. The highest root lengths (12.0, 14.3, 16.7 and 18.5 cm/plant) were recorded in groundnut grown in *Rhizobium* + *Pseudomonas* + *Bacillus* treatment. The lowest root lengths (6.4, 9.6, 12.3 and 13.7 cm/plant) were recorded at various stages of its growth (25, 50, 75 and 100 DAS) in the crops grown without Plant growth promoting rhizobacteria treatment.

Number of root nodules (nodules/plant)

The results on the effect of Plant growth promoting rhizobacteria on number of root nodules per plant of groundnut at various stages of its growth (25, 50, 75 and 100 DAS) are shown in Table 3. The highest numbers of root nodules (45.0, 65.0, 92.0 and 112.0 per plant) were recorded in groundnut crop grown in *Rhizobium* + *Pseudomonas* + *Bacillus* treatment. The lowest numbers of root nodules (10.0, 21.0, 34.0 and 48.0 per plant) were recorded at various stages of its growth (25, 50, 75 and 100 DAS) in crop grown without Plant growth promoting rhizobacteria treatment.

Total leaf area (cm²/plant)

The results on the effect of Plant growth promoting rhizobacteria fertilizer on total leaf area (cm²/plant) of groundnut at various stages of its growth (25, 50, 75 and 100 DAS) are shown in Table 4. The highest total leaf areas (5.70, 7.09, 11.02 and 13.73 cm²/plant) were recorded in groundnut crop grown in *Rhizobium* + *Pseudomonas* + *Bacillus* treatment. The lowest total leaf area (4.38, 5.76, 7.82 and 9.92 cm²/plant) was recorded at various stages of its growth (25,

50, 75 and 100 DAS) in the crops grown without Plant growth promoting rhizobacteria treatment.

Fresh weight of plant (g/plant)

The effect of Plant growth promoting rhizobacteria on fresh weight (g/plant) of groundnut crop at various stages of its growth (25, 50, 75 and 100 DAS) is shown in Table 5. The highest fresh weights (20.48, 41.89, 128.89 and 232.19 g/plant) were recorded in groundnut crop grown with *Rhizobium* + *Pseudomonas* + *Bacillus* treatment. The lowest fresh weight of plant (9.71, 18.87, 42.39 and 98.20 g/plant) at various 25, 50, 75 and 100 DAS, were recorded in the crops grown without Plant growth promoting rhizobacteria treatment.

Dry weight of plant (g/plant)

The results on the effect of various treatment of plant growth promoting rhizobacteria on dry weight (g/plant) of groundnut crop at various stages of its growth (25, 50, 75 and 100 DAS) are shown in Table 6. The highest dry weights of plant (6.83, 13.96, 42.96 and 72.40 g/plant) were recorded in groundnut crop grown with *Rhizobium* + *Pseudomonas* + *Bacillus* treatment. The lowest dry weights (3.24, 6.29, 14.13 and 32.75 g/plant) of plant at 25, 50, 75 and 100 DAS were recorded in the crops grown without plant growth promoting rhizobacteria treatment.

Yield parameters

The effect of application of various treatment of plant growth promoting rhizobacteria on the yield parameters of groundnut are shown in Table 7. The highest number of pods (48.0 per plant), number of seeds (93.0 per pods), hundred seed weight (66.4 g) were recorded in the crop grown

with *Rhizobium* + *Pseudomonas* + *Bacillus* treatment. The lowest number of pods (23.0 per plant), number of seed (45.0 per pot), hundred seed weight (40.5 g/plant) were recorded in the crops grown in the control plants which are grown without plant growth promoting rhizobacteria application.

Soil Properties

The results on the effect of various treatment of plant growth promoting rhizobacteria on physico-chemical analysis of soil are shown in Table 8.

The control soil has the pH value of 7.55 and EC values of 0.40 dS/m. The macronutrients such as available N (115.6 kg/acre), available P (4.5 kg/acre) and available K (100.5 kg/acre) were recorded. The values of micronutrients such as available Zn (4.20 kg/acre), Cu, (0.18 kg/acre), Fe (7.15 kg/acre) and Mn (4.50 kg/acre) were recorded.

The pH values of soil (7.0) showed the variation among the fertilizer applied soil. The highest values of EC 0.99 dSm⁻¹ available

N 167.9 kg/acre, P (9.8 kg/acre), K (154.7 kg/acre), Zn (6.73 kg/acre), Cu (0.61 kg/acre), Fe (9.10 kg/acre) and Mn (6.03 kg/acre) were recorded in the combined inoculation of plant growth promoting rhizobacteria.

Root length and shoot length

In the pot culture experiment, the highest root and shoot growth of groundnut were observed in the combined inoculation of plant growth promoting rhizobacteria when compared with control as well as with other treatment. The observed growth promotion is based on several parameters

including increase in root length, shoot length, branching nodulation in legume crop, dry biomass, yield and seed weight. PGPR have been demonstrated to increase growth and productivity of many commercial crops, cucumber (Maleki *et al.*, 2010),

Root nodules

Legume nodules are spherical or cylindrical growth formed in the plant roots as a result of an infection by bacteria (Streeter, 1995). Only certain bacteria, some blue green algae and leguminous plants can fix the atmosphere nitrogen to the root nodule (Jain, 2000). In this experiment, the highest nodule number of groundnut was observed in combined inoculation of plant growth promoting rhizobacteria application when compared to control as well as with other treatments. Co inoculation of some *Pseudomonas* and *bacillus* strains with effective *Rhizobium sps* stimulated growth, nodulation and nitrogen fixation findings of chickpea (Mohammadi *et al.*, 2010).

Total leaf area

Leaf area is an important part of the plant responsible for interception and conversion of solar energy (Sarkar *et al.*, 1995). Total leaf area is the index of rate of photosynthesis which reflects the crop production. The highest total leaf area was recorded in groundnut crop in the application of combined inoculation of plant growth promoting rhizobacteria. The lowest total leaf area was recorded in plants grown in control pots. Inoculation of PGPR species could increase the growth attributes like leaf area, chlorophyll content and consequently, the total biomass of the musa plantlets under nitrogen free hydroponics as compared to the uninoculated control (Mia *et al.*, 2010).

Table.1 Effect of various application of Plant growth promoting rhizobacteria on shoot length (cm/plant) at different stages of growth groundnut (*Arachis hypogaea* L.)

Treatments	Age of the plant in days			
	25	50	75	100
Control (T ₀)	5.5 ± 0.16	12.1 ± 0.36	23.1 ± 0.69	30.7 ± 0.92
Rhizobium (T ₁)	6.0 ± 0.18	14.2 ± 0.43	26.5 ± 0.79	33.1 ± 0.99
Pseudomonas (T ₂)	6.7 ± 0.20	16.4 ± 0.49	28.4 ± 0.85	36.2 ± 1.09
Bacillus (T ₃)	7.1 ± 0.21	18.3 ± 0.55	30.1 ± 0.90	39.4 ± 1.18
Rhizobium + Pseudomonas (T ₄)	7.8 ± 0.23	20.4 ± 0.61	32.4 ± 0.97	41.5 ± 1.24
Rhizobium + Bacillus (T ₅)	8.4 ± 0.25	22.1 ± 0.66	34.2 ± 1.03	43.0 ± 1.29
Pseudomonas + Bacillus (T ₆)	8.9 ± 0.27	24.4 ± 0.73	36.0 ± 1.08	45.3 ± 1.36
Rhizobium + Pseudomonas+ Bacillus (T ₇)	9.4 ± 0.28	25.7 ± 0.77	38.2 ± 1.15	47.8 ± 1.43

± Standard deviation

Table.2 Effect of application of various treatment of Plant growth promoting rhizobacteria on root length (cm/plant) of groundnut (*Arachis hypogaea* L.) at different growth stages

Treatments	Age of the plant in days			
	25	50	75	100
Control (T ₀)	6.4 ± 0.19	9.6 ± 0.29	12.3 ± 0.37	13.7 ± 0.41
Rhizobium (T ₁)	7.3 ± 0.22	10.2 ± 0.31	12.9 ± 0.39	14.6 ± 0.44
Pseudomonas (T ₂)	8.8 ± 0.26	10.9 ± 0.33	13.4 ± 0.40	15.5 ± 0.46
Bacillus (T ₃)	9.5 ± 0.28	11.6 ± 0.35	14.1 ± 0.42	16.0 ± 0.48
Rhizobium + Pseudomonas (T ₄)	10.0 ± 0.30	12.1 ± 0.36	14.8 ± 0.44	16.6 ± 0.50
Rhizobium + Bacillus (T ₅)	10.7 ± 0.32	12.9 ± 0.39	15.4 ± 0.46	17.4 ± 0.52
Pseudomonas + Bacillus (T ₆)	11.3 ± 0.34	13.5 ± 0.40	16.0 ± 0.48	18.0 ± 0.54
Rhizobium + Pseudomonas + Bacillus (T ₇)	12.0 ± 0.36	14.3 ± 0.43	16.7 ± 0.50	18.5 ± 0.55s

± Standard deviation

Table.3 Effect of application of various treatment Plant growth promoting rhizobacteria on number of root nodules/plant of groundnut (*Arachis hypogaea* L.)

Treatments	Age of the plant in days			
	25	50	75	100
Control (T ₀)	10.0 ± 0.30	21.0 ± 0.63	34.0 ± 1.02	48.0 ± 1.44
Rhizobium (T ₁)	15.0 ± 0.45	28.0 ± 0.84	42.0 ± 1.26	57.0 ± 1.71
Pseudomonas (T ₂)	21.0 ± 0.63	35.0 ± 1.05	51.0 ± 1.53	66.0 ± 1.98
Bacillus (T ₃)	28.0 ± 0.84	41.0 ± 1.23	59.0 ± 1.77	74.0 ± 2.22
Rhizobium + Pseudomonas (T ₄)	32.0 ± 0.96	48.0 ± 1.44	67.0 ± 2.01	82.0 ± 2.46
Rhizobium + Bacillus (T ₅)	37.0 ± 1.11	54.0 ± 1.62	76.0 ± 2.28	92.0 ± 2.76
Pseudomonas + Bacillus (T ₆)	42.0 ± 1.26	60.0 ± 1.80	84.0 ± 2.52	103.0 ± 3.09
Rhizobium +Pseudomonas+ Bacillus (T ₇)	48.0 ± 1.44	65.0 ± 1.95	92.0 ± 2.76	112.0 ± 3.36s

± Standard deviation

Table.4 Effect of application of various treatment Plant growth promoting rhizobacteria on total leaf area (cm²/plant) of groundnut (*Arachis hypogaea* L.)

Treatments	Age of the plant in days			
	25	50	75	100
Control (T ₀)	4.38 ± 0.13	5.76 ± 0.17	7.82 ± 0.23	9.92 ± 0.30
Rhizobium (T ₁)	4.54 ± 0.14	5.94 ± 0.18	8.24 ± 0.25	10.46 ± 0.31
Pseudomonas (T ₂)	4.71 ± 0.14	6.14 ± 0.18	8.73 ± 0.26	11.00 ± 0.33
Bacillus (T ₃)	4.98 ± 0.15	6.34 ± 0.19	9.20 ± 0.	11.57 ± 0.35
Rhizobium +Pseudomonas (T ₄)	5.17 ± 0.15	6.50 ± 0.20	9.72 ± 0.28	12.13 ± 0.36
Rhizobium + Bacillus (T ₅)	5.34 ± 0.16	6.69 ± 0.20	10.16 ± 0.30	12.67 ± 0.38
Pseudomonas + Bacillus (T ₆)	5.52 ± 0.16	6.89 ± 0.21	10.62 ± 0.32	13.21 ± 0.40
Rhizobium + Pseudomonas + Bacillus (T ₇)	5.70 ± 0.171	7.09 ± 0.21	11.02 ± 0.33	13.73 ± 0.41

± Standard deviation

Table.5 Effect of application of various treatment of Plant growth promoting rhizobacteria on fresh weight (g/plant) of groundnut (*Arachis hypogaea* L.) at various stages of its growth

Treatments	Age of the plant in days			
	25	50	75	100
Control (T ₀)	9.71 ± 0.29	18.87 ± 0.57	42.39 ± 1.27	98.26 ± 2.95
Rhizobium (T ₁)	10.89 ± 0.33	22.39 ± 0.67	50.76 ± 1.52	115.54 ± 3.47
Pseudomonas (T ₂)	11.98 ± 0.36	25.79 ± 0.77	61.89 ± 1.86	132.82 ± 3.98
Bacillus (T ₃)	13.79 ± 0.41	28.90 ± 0.87	73.39 ± 2.20	153.78 ± 4.61
Rhizobium + Pseudomonas (T ₄)	15.62 ± 0.47	32.00 ± 0.96	84.31 ± 2.53	172.42 ± 5.17
Rhizobium + Bacillus (T ₅)	17.22 ± 0.52	35.49 ± 1.06	96.53 ± 2.90	190.12 ± 5.70
Pseudomonas + Bacillus (T ₆)	18.72 ± 0.56	38.64 ± 1.16	110.39 ± 3.31	218.19 ± 6.54
Rhizobium + Pseudomonas + Bacillus (T ₇)	20.48 ± 0.61	41.89 ± 1.26	128.89 ± 3.87	232.19 ± 6.96

± Standard deviation

Table.6 Effect of application of various treatment of Plant growth promoting rhizobacteria on dry weight (g/plant) of groundnut (*Arachis hypogaea* L.)

Treatments	Age of the plant in days			
	25	50	75	100
Control (T ₀)	3.24 ± 0.09	6.29 ± 0.19	14.13 ± 0.42	32.75 ± 0.98
Rhizobium (T ₁)	3.63 ± 0.11	7.46 ± 0.22	16.75 ± 5.58	38.51 ± 1.15
Pseudomonas (T ₂)	3.99 ± 0.12	8.60 ± 0.26	20.63 ± 0.62	44.27 ± 1.33
Bacillus (T ₃)	4.60 ± 0.14	9.63 ± 0.29	24.46 ± 0.73	51.26 ± 1.54
Rhizobium + Pseudomonas (T ₄)	5.21 ± 0.16	10.67 ± 0.32	28.10 ± 0.843	57.47 ± 1.72
Rhizobium + Bacillus (T ₅)	5.74 ± 0.17	11.83 ± 0.35	32.18 ± 0.96	63.37 ± 1.90
Pseudomonas + Bacillus (T ₆)	6.24 ± 0.19	12.88 ± 0.39	36.80 ± 1.10	72.73 ± 2.18
Rhizobium + Pseudomonas + Bacillus (T ₇)	6.83 ± 0.20	13.96 ± 0.42	42.96 ± 1.29	77.40 ± 2.32

± Standard deviation

Table.7 Effect of application of various treatment of Plant growth promoting rhizobacteria on yield parameters on groundnut (*Arachis hypogaea* L.)

Treatments	Number of pods/plant	Number of seeds/plant	100 seed weight/plant
Control (T ₀)	23.0 ± 0.69	45.0 ± 1.35	45.8 ± 1.37
Rhizobium (T ₁)	28.0 ± 0.84	53.0 ± 1.59	48.9 ± 1.47
Pseudomonas n (T ₂)	30.0 ± 0.90	60.0 ± 1.80	51.0 ± 1.53
Bacillus (T ₃)	33.0 ± 0.99	65.0 ± 1.95	53.9 ± 1.62
Rhizobium + Pseudomonas (T ₄)	37.0 ± 1.11	72.0 ± 2.16	55.8 ± 1.67
Rhizobium +Bacillus (T ₅)	40.0 ± 1.20	81.0 ± 2.43	59.0 ± 1.77.
Pseudomonas + Bacillus (T ₆)	45.0 ± 1.35	86.0 ± 2.58	62.1 ± 1.86
Rhizobium +Pseudomonas + Bacillus (T ₇)	48.0 ± 1.44	93.0 ± 2.79	66.4 ± 1.99

± Standard deviation

Table.8 Physico-chemical analysis of experimental soil (before sowing and after harvesting)

Treatment	pH	EC (dSm ⁻¹)	Available N (mg/kg)	Available P (mg/kg)	Available K (mg/kg)	Available Zn (mg/kg)	Available Cu (mg/kg)	Available Fe (mg/kg)	Available Mn (mg/kg)
Before sowing									
T ₀	7.55	0.40	115.6	4.5	100.5	4.20	0.18	7.15	4.50
After Harvesting									
T ₁	7.52	0.56	125.6	5.5	105.5	5.15	0.24	8.15	5.20
T ₂	7.50	0.77	139.7	6.2	120.0	5.78	0.35	8.19	5.23
T ₃	7.32	0.95	145.2	9.4	132.5	6.37	0.48	8.26	5.38
T ₄	7.50	0.93	154.3	8.2	140.0	6.49	0.59	8.18	5.52
T ₅	7.42	0.92	152.4	8.0	139.8	6.12	0.52	8.58	5.61
T ₆	7.41	0.97	159.7	8.3	145.0	6.01	0.53	8.53	5.63
T ₇	7.00	0.99	167.9	9.8	154.7	6.73	0.61	9.10	6.03

Fresh weight and dry weight

The fresh weight and dry weight are mainly based on their growth performance of a particular crop. A plant can grow vigorously if it contains much amount of

fresh weight and dry weight. In the present study, the application of combined inoculation of plant growth promoting rhizobacteria increased fresh weight and dry weight of crop plants at the highest level when compared to control as well as with

other treatment. The highest fresh weight and dry weight were recorded in 100 day old plants followed by 75, 50 and 25 DAS.

The root and shoot dry weight significantly increased by combined inoculation of seed with *Mesorhizobium sp*, *Pseudomonas aeruginosa*, *Bacillus megaterium* and *A.chroococcum* respectively (Verma *et al.*, 2013). The effect of the mixed inoculation of the *Rhizobium sp*, *Pseudomonas fluorescens* and *Bacillus megaterium* significantly increases the root and shoot growth when compared to uninoculated on green gram (Maqshoof Ahmad *et al.*, 2012; Anandaraj *et al.*, 2010; Mishra *et al.*, 2010).

Yield parameters

In this experiment, the highest yield parameters (number of pods per plant, number of seeds per plant, 100 seed weight and yield) were registered in the crop grown under the inoculation of plant growth promoting rhizobacteria (control, *Rhizobium*, *Pseudomonas*, *Bacillus*, *Rhizobium* + *Pseudomonas*, *Rhizobium* + *Bacillus*, *Pseudomonas* + *Bacillus*, and *Rhizobium* + *Pseudomonas* + *Bacillus*.) treatment. Almost forty five per cent yield increase was observed in combined inoculation of plant growth promoting rhizobacteria treatment. Many studies have recently shown that inoculation with some plant growth promoting bacteria, increased growth and yield in great number in plants including legumes (Pirlak and Kose, 2009; Shaharoon *et al.*, 2006; Tilak *et al.*, 2006).

Soil properties

The analysis of soil applied with fertilizer showed that it has all kinds of nutrient needed for the better growth of the crop. The soil properties such as pH, EC, available nitrogen, phosphorus, potassium, iron, zinc,

copper and manganese were found to vary in the soil treated with plant growth promoting rhizobacteria application. An increase in micro and macronutrient content were observed with the combined inoculation of plant growth promoting rhizobacteria treatment. Soil bacteria have been used in crop production for decades. The main functions of these bacteria are (1) to supply nutrients to crops; (2) to stimulate plant growth, e.g., through the production of plant hormones; (3) to control or inhibit the activity of plant pathogens; (4) to improve soil structure; and (5) bioaccumulation or microbial leaching of inorganic (Brierley 1985; Ehrlich 1990). Free-living soil bacteria beneficial to plant growth are usually referred to as Plant Growth Promoting Rhizobacteria (PGPR), capable of promoting plant growth by colonizing the plant root (Kloepper and Schroth 1978; Cleyet-Marcel *et al.*, 2001).

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