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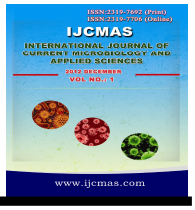

Original Research Article

Comparative effect on bacterial biofertilizers on growth and yield of green gram (*Phaseolus radiata* L.) and cow pea (*Vigna siensis* Edhl.)

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KEYWORDS

Bacterial biofertilizers; seed inoculation; morphological and bio-chemical parameters ; Chromatographic technique

A B S T R A C T

The comparative effect of bacterial biofertilizers such as *Rhizobium*, Phosphobacteria and *Azozpirillum* on growth and yield of green gram (*Phaseolus radiata* L.) and cowpea (*Vigna siensis* Edhl.) was studied. The bacteria were isolated from the soil samples and identified by staining and biochemical tests. The seeds were inoculated with bacterial biofertilizers with various treatments and showed in sterile polythene bag containing sterilized soil. After 65 days of plant growth, the morphological and bio-chemical parameters of cowpea were increased in combined inoculation of *Rhizobium*, Phosphobacteria and *Azospirillum* than green gram plants.

Introduction

Biofertilizers are the green manure and organics. Biofertilizers are carrier-based inoculants containing cells of efficient strains of specific microorganisms (namely bacteria) used by farmers for enhancing the productivity of the soil by fixing atmospheric nitrogen or by solubilizing soil phosphate or by stimulating plant growth for synthesis of growth promoting substances.

Biofertilizers play a main key role for selective adsorption of immobile (P, Zn, Cu) and mobile (C, S, Ca, K, Mn, Cl, Br, and N) elements to plants (Tinker, 1984). The rhizosphere bacteria secrete growth substances and secondary metabolic, which contribute to seed germination and plant growth (Subba Rao, 1982, 2002; Dwivedi, 1989). In recent years, free – living bacteria (*Azotobacter*), associate (*Azospirillum*) and symbiotic (*Rhizobium*) bacteria and phosphate solubilizing one (*Bacillus megaterium, B. polymyxa* and *Ps. Striata*) are gaining much popularity.

Such practices are being encouraged to save the chemical fertilizers natural economy and the environment.

Materials and Methods

Polythene bag method was conducted to study about the comparative effect of bacterial biofertilizers on pulse crops like green gram (*Phseolus radiata* L.) and cowpea (*Vigna siensis* Edhl.). Bacterial biofertilizers such as *Rhizobium*, Phosphobacteria and *Azospirillum* were isolated from the root nodule and soil samples by plating technique and identified according to Bergey's manual of Determinative Bacteriology (9th Edition).

Seed inoculation was done by various alone treatments like Phosphobacteria (T1), *Azospirillum* (T2) and *Rhizobium* (T3), dual inoculations like Phosphobacteria and *Azospirillum* (T4), *Rhizobium* and *Azospirillum* (T5), *Rhizobium* and Phosphobacteria (T6) and combined inoculations of *Rhizobium*, Phosphobacteria and *Azospirillum* (T7). Control was also maintained without biofertilizers.

The seeds were sowed in sterile polythene bags containing sterile soil samples.

After 65 days of sowing, the morphological and bio-chemical parameters of green gram and cowpea were analyzed. The morphological parameters like length of plant, number of leaves, breadth of leaves, length of leaves, shoot length, number of flowers, root length, number of nodules and number of pods were analyzed. The bio-chemical parameters such as chlorophyll, protein, carbohydrate, total free amino acids, nitrogen, ash, inorganic phosphorus, reducing sugar, alkaline phosphatase, glutamate dehydroganase were analyzed both control and treated plants of green gram and cowpea.

The amino acids contents of green gram and cowpea samples were separated by two dimensional paper chromatography. In this, 20µl of each sample were spotted on the whattmann No.1 chromatographic paper and sheet was mounted on the metal frame. The papers were placed in solvent 1 containing butanol, glacial acetic acid and water (12:3:5). After running in first solvent, the papers again placed in solvent 2 containing phenol and water. The papers rapidly dipped in ninhydrin reagent and colour was developed by heating at 105ºC for 2-3 minutes. Then Rf values were measured (Plummer, 1998)

Lipids were separated by Thin Layer Chromatography techniques. In this, an aqueous phase of silica gel slurry was poured on the surface of the glass plates of 250µm thickness. The plates were activated by heating 110ºC for 1 hour and allowed to cool in room temperature. 20µl of each sample was spotted onto the plates and placed in solvent containing petroleum ether, diethyl ether and glacial acetic acid (80:20:1) and run the chromatogram. The spot was visualized by spraying the plates with 50% v/v sulfuric acid followed by heating the oven at 110ºC for 10 minutes.

Then Rf values were measured (Plummer, 1998).

$$Rf = \frac{\text{Distance moved by solute}}{\text{Distance moved by solvent}}$$

From the data, statistical analysis such as Mean (M), standard deviation (SD) and Standard error (SE) were also calculated (Smith's Statistical package, Version 2.5, 2001).

Results and Discussion

The seed inoculation with bacterial biofertilizers like *Rhizobium*, Phosphobacteria and *Azospirillum* at various treatments were significantly increased in plant growth and yield of green gram and cowpea plants. The number of leaves, leaf area (length and breadth), shoot length, root length, number of nodules, total length of plants at 65 days after sowing was significantly more with there combined treatment with *Rhizobium*, Phosphobacteria and *Azospirillum* inoculated plants of cow pea than green gram (Table 1 and 2). The yield concepts such as number of flowers and number of pods were increased in the combined treatments with *Rhizobium*, Phosphobacteria and *Azospirillum* inoculated plants of cowpea than green gram (Tables 3 and 4). This was well agreed with previous findings of Gaur and Agarwal (1989), Tilak (1991) and Vasudevan *et al* (2002).

The bio-chemical parameters such as chlorophyll, protein, carbohydrate, total free amino acids, inorganic phosphorus, nitrogen were increased in treated with combined inoculation of bacterial biofertilizers (T7) of cowpea than green gram (Table5 and 6). This was well correlated with earlier studies on *Vigna mungo* L. (Mohan et al., 1994; Shukla and Gupta, 1964). Increase in ascorbic acid, reducing sugar content were observed in combined inoculation of bacterial biofertilizers (T7) of cowpea plants than green gram. Activity of enzymes like alkaline phosphatase and glutamate dehydrogenase were higher in combined inoculation of cow pea plants (Table 7 and 8). All the parameters like morphological and bio-chemical parameters of cowpea treated with bacterial biofertilizers in dual and combined inoculations were higher than green gram. It was accepted with previous reports of Balamurugan and Gurusejaran (1996), Agarwal and Tilak (1989) and Gupta *et al* (1992).

By employing two dimensional paper chromatography techniques, the amino acid contents of cowpea plants were higher in combined treatments and Rf values were 0.90 than green gram. The lipid contents of cowpea plants were separated by TLC technique and their Rf values were 0.98 in combined inoculation of bacterial biofertilizers than green gram (Fig 1 and 2).

Table. 1 Effect of morphological parameters of cowpea plants inoculated with bacterial biofertilizers

Treatments	Parameters in cm						
	Number of leaves/plant	Length of leaves	Breadth of leaves	Length of plant	Shoot length	Root length	Total length of plant
Control	6.8	5.4	2.5	48.9	24.6	9.0	57.9
Phosphobacteria	7.6	6.3	2.6	53.2	25.4	12.2	75.4
Azospirillum	9.0	5.6	2.9	48.2	26.6	11.4	59.6
Rhizobium	9.2	6.2	2.8	53.2	25.8	11.0	64.2
Phosphobacteria+*Azospirillum*	8.4	6.4	3.1	50.0	28.4	12.8	62.8
Rhizobium + *Azospirillum*	9.6	5.7	2.6	52.0	30.0	14.6	66.6
Rhizobium+Phophobacteria	9.4	6.4	3.1	54.6	28.8	14.6	69.2
Rhizobium +Phophobacteria +*Azospirillum*	10.6	6.8	4.0	55.0	31.0	17.2	72.2

Table. 2 Effect of morphological parameters of green gram plants inoculated with bacterial biofertilizers

Treatments	Parameters in cm						
	Number of leaves/plant	Length of leaves	Breadth of leaves	Length of plant	Shoot length	Root length	Total length of plant
Control	7.0	4.8	2.1	20.9	15.9	4.0	21.5
Phosphobacteria	7.4	5.1	2.4	21.6	19.4	4.9	25.8
Azospirillum	7.4	5.4	2.1	21.3	20.2	5.9	25.7
Rhizobium	7.6	5.7	2.8	31.0	23.4	6.6	37.6
Phosphobacteria+*Azospirillum*	8.0	5.1	2.9	34.2	21.8	7.1	41.3
Rhizobium + *Azospirillum*	8.8	4.5	2.3	34.2	20.8	6.4	40.8
Rhizobium+Phophobacteria	8.8	5.8	2.4	34.4	22.6	7.8	41.8
Rhizobium +Phophobacteria +*Azospirillum*	9.0	6.6	2.4	35.2	25.2	7.9	43.5

Table. 3 Effect on yield concepts of cow pea plants inoculated with bacterial biofertilizers

Treatments	Parameters in cm		
	Number of nodules/plant	Number of flowers/ plant	Number of pods/plant
Control	18.4	2.4	2.0
Phosphobacteria	20.0	2.4	2.4
Azospirillum	21.2	2.8	3.4
Rhizobium	18.2	2.8	3.0
Phosphobacteria+*Azospirillum*	21.4	3.4	3.2
Rhizobium + *Azospirillum*	22.4	4.2	3.2
Rhizobium+Phophobacteria	21.8	3.0	4.0
Rhizobium +Phophobacteria +*Azospirillum*	23.2	4.8	4.2

Table. 4 Effect on yield concepts of green gram plants inoculated with bacterial biofertilizers

Treatments	Parameters in cm		
	Number of nodules/plant	Number of flowers/ plant	Number of pods/plant
Control	8.4	1.6	1.2
Phosphobacteria	9.0	1.8	2.2
Azospirillum	12.0	1.7	3.0
Rhizobium	12.2	1.8	3.0
Phosphobacteria+*Azospirillum*	12.0	1.8	3.0
Rhizobium + *Azospirillum*	18.2	2.0	3.2
Rhizobium+Phophobacteria	21.0	2.0	3.6
Rhizobium +Phophobacteria +*Azospirillum*	22.2	4.2	4.0

Table. 5 Effect of biochemical parameters of cowpea plants inoculated with bacterial biofertilizers

Treatments	Parameters in mg/g					
	Chlorophyll	Protein	Carbohydrate	Amino acids	Inorganic phosphorus	Nitrogen
Control	1.06	0.92	13.14	3.6	2.02	2.25
Phosphobacteria	1.82	0.98	14.80	5.6	2.13	2.84
Azospirillum	1.84	0.97	14.80	8.67	2.32	3.45
Rhizobium	1.86	1.0	15.11	9.60	2.18	2.84
Phosphobacteria+*Azospirillum*	2.30	1.0	15.27	12.24	2.72	3.62
Rhizobium + *Azospirillum*	2.34	1.10	15.51	13.37	2.74	3.62
Rhizobium+Phophobacteria	2.51	1.10	15.59	13.37	2.66	3.82
Rhizobium +Phophobacteria +*Azospirillum*	4.04	1.44	15.74	18.36	3.52	4.80

Table. 6 Effect of biochemical parameters of cowpea plants inoculated with bacterial biofertilizers

Treatments	Parameters in mg/g					
	Chlorophyll	Protein	Carbohydrate	Amino acids	Inorganic phosphorus	Nitrogen
Control	0.70	0.25	11.0	2.25	2.08	0.68
Phosphobacteria	1.06	0.30	14.01	5.10	2.26	0.88
Azospirillum	1.37	0.27	14.80	5.60	2.13	0.88
Rhizobium	1.57	0.33	14.80	7.60	2.26	0.68
Phosphobacteria+*Azospirillum*	1.60	0.62	15.11	9.69	2.58	1.07
Rhizobium + *Azospirillum*	1.91	0.54	15.27	9.18	2.45	1.66
Rhizobium+Phophobacteria	1.99	0.56	15.51	11.73	2.64	1.86
Rhizobium +Phophobacteria +*Azospirillum*	2.21	1.17	15.57	11.75	2.90	2.64

Table. 7 Effect of biochemical parameters of cowpea plants inoculated with bacterial biofertilizers

Treatments	Parameters in mg/g				
	Ascorbic acid	Reducing sugar	Ash	Alkaline phosphatase	Glutamate dehydrogenase
Control	0.85	1.62	30	0.29	30
Phosphobacteria	0.95	1.65	45	0.37	50
Azospirillum	0.90	1.90	45	0.35	40
Rhizobium	0.85	1.78	45	0.39	40
Phosphobacteria+*Azospirillum*	2.34	3.33	80	0.45	50
Rhizobium + *Azospirillum*	2.23	2.01	80	0.58	50
Rhizobium+Phophobacteria	2.45	3.83	85	0.70	90
Rhizobium +Phophobacteria +*Azospirillum*	2.56	4.50	90	0.91	100

Table. 8 Effect of biochemical parameters of cowpea plants inoculated with bacterial biofertilizers

Treatments	Parameters in mg/g				
	Ascorbic acid	Reducing sugar	Ash	Alkaline phosphatase	Glutamate dehydrogenase
Control	0.12	1.80	30	0.16	20
Phosphobacteria	0.42	1.90	45	0.31	60
Azospirillum	0.30	3.40	40	0.37	40
Rhizobium	0.85	1.90	40	0.25	50
Phosphobacteria+*Azospirillum*	2.07	3.80	35	0.62	60
Rhizobium + *Azospirillum*	2.10	3.30	35	0.47	40
Rhizobium+Phophobacteria	2.15	3.70	50	0.62	60
Rhizobium +Phophobacteria +*Azospirillum*	2.37	3.95	70	0.82	60

Figure 1. Analysis of amino acids and lipids in cowpea plants by chromatography technique.

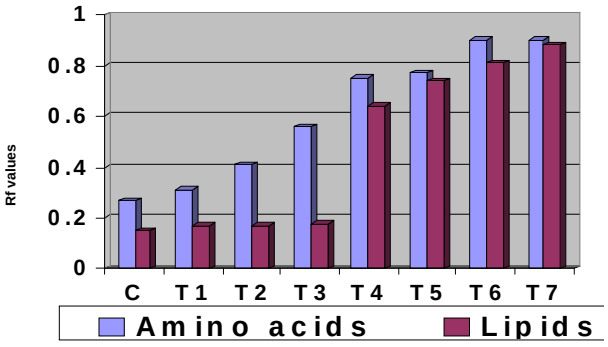


Figure 2. Analysis of amino acids and lipids in green gram plants by chromatography technique**.**

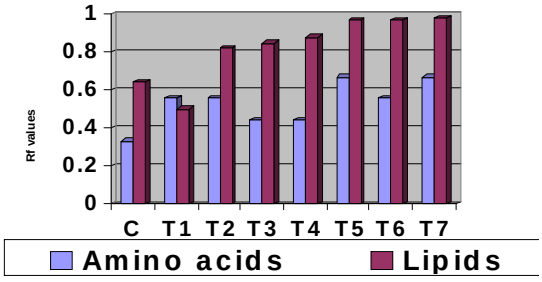


Figure 3. Statistical analysis of morphological parameters of cowpea plants

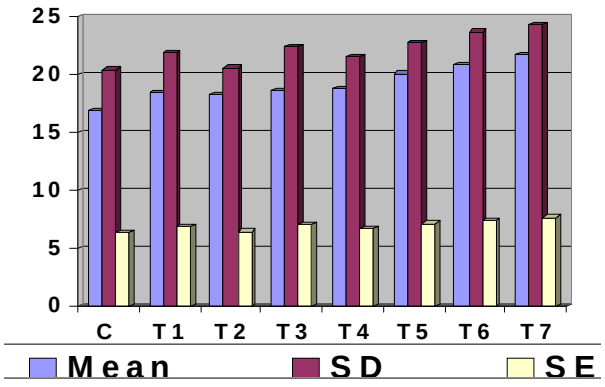


Figure 4. Statistical analysis of morphological parameters of green gram plants

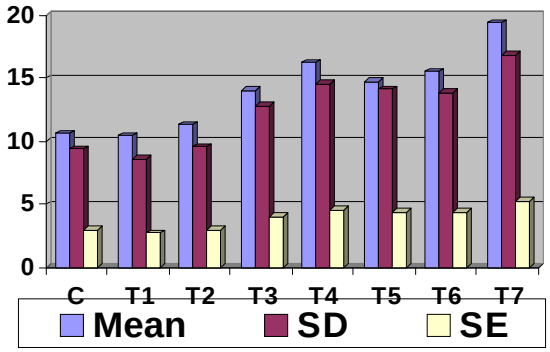


Figure 5. Statistical analysis of biochemical parameters of cowpea plants

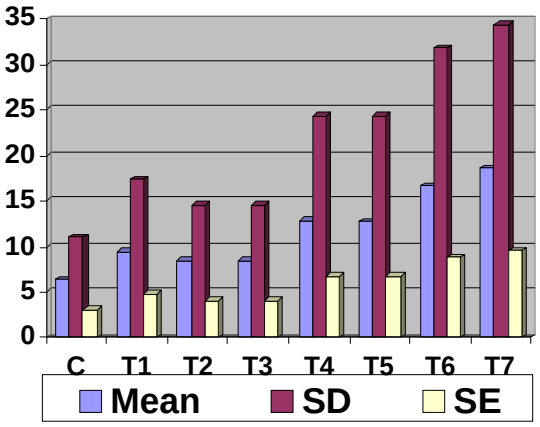
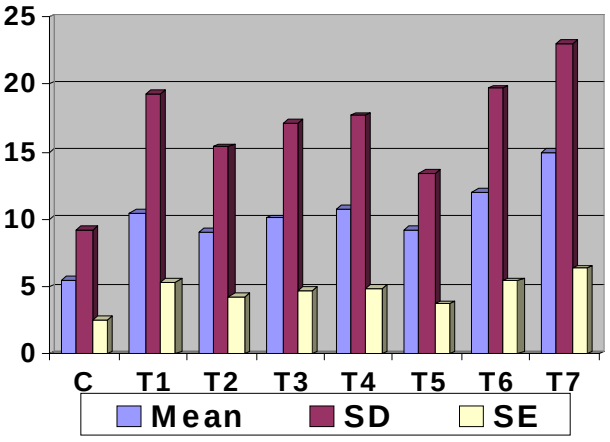


Figure 6. Statistical analysis of biochemical parameters of green gram plants



Statistical analysis studied for morphological parameters of cowpea plants were higher. When compared to control plants of cowpea, Mean, Standard Deviation and Standard error were 22.83, 23.63 and 0.45 respectively on 65 DAS (Fig 3 and 4). Bio-chemical parameters of cowpea, Mean, Standard Deviation and Standard error were 19.05, 34.21 and 0.4 respectively on 65 DAS (Fig 5 and 6). Thus, using bacterial members such as *Rhizobium*, Phosphobacteria and *Azospirillum* as biofertilizers, which improve the growth and yield of pulse crops and also reduce the use of chemical fertilizers.

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