International Journal of Advanced Research in Biological Sciences
www.ijarbs.com

Research Article

A Study on various treatments of bacterial biofertilizers on the growth of *Cajanus cajan* L.

S.Prabha[1],T.Sivakumar[1]* and M.Ravikumar[2]

[1]PG and Research Department of Microbiology, Kanchi Shri Krishna College of Arts and Science, Kilambi, Krishnapuram, Kancheepuram -631 551, Tamil Nadu, India

[2]Department of Plant Biology and Biotechnology, Government Arts College, Nandhanam, Chennai, Tamil Nadu, India

*Corresponding author e-mail: *shiva.fungi@gmail.com*

Abstract

The present investigation was carried out to study the effect of bacterial Biofertilizers on *Cajanus cajan* L. plant. Bacterial biofertilizers like *Rhizobium* sp., Phosphobacteria, *Azospirillm* sp. and *Azotobacter* sp. were isolated from the soil of agricultural crops by employing plating techniques. The microbial inoculants coated seed were sowed in pot containing sterile soil samples. Controls were also maintained without a bacterial biofertilizers. After 45 days of sowing, the plant growth parameters like morphological and Bio-chemical parameters were analyzed in *Cajanus cajan* plants. 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, total length of plants were increased in combined inoculation of *Rhizobium* sp., Phosphobacteria, *Azospirillum* sp. and *Azotobacter* sp. in *Cajanus cajan* L. than dual inoculations and control plants. io-Chemical parameters like Chlorophyll content, Protein, Carbohydrate, Total free amino acids, Nitrogen, ash content, Inorganic phosphorus, Reducing sugars, Alkaline phosphatase and Glutamate dehydrogenase, were also increased in combined treatment of *Rhizobium* sp., Phosphobacteria, *Azospirillum* sp. and *Azotobacter* sp. plants of *Cajanus cajan* L. than dual inoculation and control plants.

Keywords: Bacterial Biofertilizers, *Cajanus cajan* L. plant, morphological, Bio-chemical parameters.

Introduction

The population of our country is increasing in geometric progression while food production is increasing in arithmetic progression. To meet the needs of people, several methods are being adapted to increase the food production; one such thing is supplementing the deficient nutrients to a plant and to increase its yield by addition of chemicals and biofertilizers. Biofertilizers are becoming more and more popular, since it is cheap and renewable one, and will not cause any pollution. One of the major concerns in today's world is pollution and contamination free environment. The use of chemical fertilizers and pesticides has caused tremendous effect to the environment. Biofertilizers will help to solve such problems as increased salinity of soil and chemical run off from the agricultural field.

In recent years, use of microbial inoculants as a source of biofierlizer has become a hope for most of the countries, as far as economical and environmental view points are concerned. Therefore, in developing countries like India, it can solve the problem of high cost of fertilizers and help in saving the economy of the country. In Rhizosphere bacteria secrete growth substance and secondary metabolics which contribute to seed germination and plant growth. (Subba Raw, 1982) Dwivedi *et al*., 1989). On application of biofertilizers, increase in rice yield ranges between 10 – 45 % kg of Nitrogen is left over in the soil which in turn is used took subsequent crops (Venkatraman, 1972), moreover benefits from algalization is about 25 – 30 KgN/ha Maize plans (Subba Rao, 1993)

Rewari (1984, 1985) reported that the increase in their grain yield over the control of Cajanes cajan, Cchickpea and Black gram plants. Subba Rao and Tilak (1977) studied that the increase the yield over the control of wheat and Rice plants. Biotertilizers and Biopesticide include many types of bacteria and fungi. One group that has been extensively investigated is plant growth promoting rhizabacteria (PGPR) PGPR are root colonizing bacteria beneficial to various agricultural crops. The beneficial effects include plant growth promotion, biological control of various disease and the activation of the defense responses of the hot plant, which is termed as induced systems Resistance (ISR) were reported by Glick and Bashan (1997). Glick (1995), Tang (1994) Frommel *et al.,* (1991); Lambert and Joss (1989); Kloepper *et al.,* (1989), Van peer and Schipper (1989), Kloepper *et al.,* (1980) Granamanickam *et al.,* (2002).

Definition and types of Bioferilizers

The term “Bioferilizers” is a popular misnomer. It refers to living organisms, which augment plant nutrient supplies in one way or the other. In the strictest sense, real Bioferilizers are the green manure and organics (materials of biological origin which are added to deliver the nutrients contained them). Bioferilizers are 1. Carrier based inoculants containing cells of efficient strains of specific microorganisms (mainly bacteria) used by farmers for enhancing the productivity of the soil either by fixing atmospheric N or by solubilizing soil P or by stimulating the plant growth through synthesis of growth promoting substance 2. Blue Green Alage or Cyanobacteria and 3. Mycorrhizae.

Bioferilizers may be broadly classified into Nitrogen Bioferilizers (NB) or Phosphate Bioferilizers (PB).

Biofertilizers are known to make a number of positive contributions in agriculture. They are,

They liberate growth promoting substance and vitamins and help to maintain soil fertility.

They suppress the incidence of pathogens and control diseases.

They are cheaper, pollution free and based on renewable energy sources.

They improve soil physical properties, tilth and soil health in general.

Azotobacter and *Azospirillum*, besides supplying Nitrogen to soil, secrete antibiotics which act as pesticides.

The biofertilizers increase physico-chemical properties of soils such as soil structure, texture, water holding capacity, cation exchange capacity and pH by providing several nutrients and sufficient organic matter.

Important and contribution of Biofertilizers in Agriculture

About 80% of the population in India depends on Agriculture. Marginal farmers having less than one hectare and they till 54.6% of the total farm holding, which small farmers having 1-2 hectares holding constitute 18%. It is very difficult them to purchase and use recommended fertilizer doses at current prices. They need to exploit other less expansive nutrient sources to the maximum. In order to raise their incomes and living standards, these land holders must maximize crop effective manner. Biofertlizers based on renewable energy sources are a cost effective supplement to chemical fertilizers and can help to economies on the high investment needed for fertilizers uses as far as Nitrogen and Phosphorus are concerned.

Biofertilizers are known to make a number of positive contributions in agriculture. They are,

They liberate growth promoting substance and vitamins and help to maintain soil fertility.

They suppress the incidence of pathogens and control diseases.

They are cheaper, pollution free and based on renewable energy sources.

They improve soil physical properties, tilth and soil health in general.

Azotobacter and *Azospirillum*, besides supplying Nitrogen to soil, secrete antibiotics which act as pesticides.

The biofertilizers increase physico-chemical properties of soils such as soil structure, texture, water holding capacity, cation exchange capacity and pH by providing several nutrients and sufficient organic matter.

Among the biofertilizers used, Nitrogen fixing and Phosphate solubilizing of symbiotic bacterial members have been exploited in the pulse crops by applying them as basal dose. Likewise the plant growth promoting substances producing ability of bacterial group of *Rhizobium* sp. Phosphobacteria and *Azospirillum* sp. can also exploit to promote the growth and yield of pulse crop by using them as biofertilizers.

The present study was initiated to analyse the effect of Bacterial biofertilizers (*Rhizobium* sp., Azotobacter sp. Phosphobacteria and *Azospirillum* sp.) on different growth parameters of *Cajanus cajan* L. plants like length of plant number of leaves, breadth of leaves, length of leaves, shoot length, number of flowers, root length and total length of plants and to estimate of biological compounds such as chlorophyll, protein, carbohydrate and total free amino acids, reducing sugars, inorganic phosphorus of treated plants and control plants.

Materials and Methods

Study materials

The present investigation was undertaken to study the effect of bacterial biofertilizers on pulse crop like ***Cajanus cajan*** **L**. plants. Biofertilizers such as *Rhizobium* sp., Phosphobacteria and *Azospirillum* sp. Azotobacter sp. were isolated from soil samples and used as inoculums.

Soil selection and sterilization

Red soil was collected and it was mixed with sand in the ratio of (2:1)v/v). The sand soil mixture was sterilized at 121ºC (151bs) for one nacre for tow consecutive days.

Isolation of bacterial biofertilizers

Isolation of *Rhizobium* sp. from Root nodules:

The legume plant root was thoroughly washed with tap water to remove the adhering soil particles. The nodules were immersed in 0.1% mercuric chloride for 1 minute. The surface sterilized nodules were washed with sterile water. The nodules were homogenized and serially diluted upto 10-6 dilution. The spread plate technique was performed on YEMA plates. The plates were incubated at 37C for 24 hours.

Isolation of A*zospirillum* sp. from soil samples:

1g soil sample was serially diluted upto10-6 dilution. From each dilution, 0.1ml of sample was taken and spread plate technique was performed using Azospirillum agar. The plates were incubated for 2-3days and colony development was observed.

Isolation of A*zotobacter* sp. from soil samples:

1g soil sample was serially diluted upto10-6 dilution. From each dilution, 0.1ml of sample was taken and spread plate technique was performed using Ahbhy’s Mannitol agar. The plates were incubated for 2-3days and colony development was observed.

Isolation of Phosphobacteria from soil samples

1g soil sample was serially diluted upto10-6 diltuion. From each dilution, 0.1ml of sample was taken and spread plate technique was performed on Pikovskaya's agar. The plates were incubated for 3-4 days. Every 24 hours, the plates were checked for the presence of phosphate solubilizers, the colony that forms a clear zone.

Identification of bacteria

Identification of bacterial members was done by Gram staining, Motility test and bio-chemical tests. The isolated strains were confirmed with Bergey's Manual Of Systemic Bacterialology (Jordan,1984).

Subculturing of bacterial strains

All the isolated bacterial cultures were isolated as pure culture by subculturing them in a respective agar media. Culture of all bacteria was inoculated into specific selective agar as slants. The test tubes were incubated in a refrigerator conditions for further processing.

Identification of bacteria

Identification of bacterial members was done by Gram staining, Motility test and bio-chemical tests. The isolated strains were confirmed with Bergey's Manual Of Systemic Bacterialology (Jordan,1984).

Preparation of bacterial biofertilizers

A 100g of cane sugar was dissolved in sterile water and boiled for 15 minutes. 200g of gum arabic was added and stirred well to dissolve it. Then 200ml of bacterial culture was added into the sticker solution and mixed well. The seeds of *Cajanus cajan* L. plants were added into the slurry. The seeds were sown in the polythene bag containing sterilized soil samples.

Inoculation of bacterial biofertilizers in the soil

Sterilized soil samples and Seeds treated with bacterial biofertilizers were sowed in the soil sample containing polythene bags. Treatments were as follows

C-Control plants

T1-seeds of *Cajanus cajan* L. plants treated with Rhizobium sp.

T2-seeds of *Cajanus cajan* L. plants. treated with *Azospirillum* sp.

T3-seeds of *Cajanus cajan* L. plants. treated with *Phosphobacterium* sp.

T4-seeds of *Cajanus cajan* L. plants. treated with Rhizoium and *Azospirillum* sp.

T5-seeds of *Cajanus cajan* L.plants. treated with *Rhizobium* sp. and *Phosphobacteria* sp.

T6-seeds of *Cajanus cajan* L.plants. treated with *Azospirillum* sp. and Phosphobacteria

T7-seeds of *Cajanus cajan* L.plants. treated with *Rhizobium* sp., Phosphobacteria and *Azospirillum* sp.

T8- seeds of *Cajanus cajan* L.. plants. treated Urea and *Azospirillum* sp.

T9- seeds of *Cajanus cajan* L.plants. treated Neem Cake

After 45 days of sowing the morphological and bio-chemical parameters of *Cajanus cajan* L. plants were analysed.

Parameters analysis

Analysis Morphological parameters

Morphological parameters such as length of plant, number of leaves, breadth of leaves, length of leaves, shoot length of /plant number of flowers/plant, root length of/plant, total length of *Cajanus cajan* L. plants were recorded respectively for treated plants.

Analysis bio-chemical parameters

Estimation of biological compounds such as chlorophyll, protein, carbohydrate and total free amino acids, reducing sugars, inorganic phosphorus were also analyzed for control, treated plants with bacterial biofertilizers.

Results and Discussion

The present investigation was carried out to study the effect of bacterial biofertilizers on pulse crops like *Cajanus cajan* L.

Isolation of bacteria

Isolation of *Rhizobium* sp. from the root nodules

Rhizobium sp. colonies are white translucent, glistening, elevated, small colonies with margin mucoid colonies .Gram negative rods, Motile, *Rhizobium* sp. ferments glucose,lactose and galactose. On YEMA and *Rhizobium* media, rhizobial colonies produce gum like substances and appeared as mucoid colonies. These substances are made up water soluble extracellular polysaccharides.

Isolation of *Azospirilum* sp.

Azospirillum sp. colonies are white pellicles,2-4mm below the surface of the medium., glistening, elevated, small colonies with margin mucoid colonies.Gram negative rods, Motile, Ferments glucose, fructose and sucrose.

Isolation of *Azotobacter* sp.

On Pikovskaya's agar, the colony morphology is transparent zone of clearing around the colonies ,Gram negative bacillus. On Pikovskaya's agar, the colony morphology is transparent zone of clearing around the colonies ,Gram negative bacillus.

Field experiment for growth of *Cajanus cajan* L. Plants.

The bacterial biofertilizers of with *Rhizobium* sp., Azotobacter sp. Phosphobacteria and *Azospirillum* sp. inoculated plants showed increase in the growth of *Cajanus cajan* L. when compared with control plants. All the parameters like morphological and bio-chemical parameters increased in dual inoculated plants and more in FYM, *Rhizobium* sp., Azotobacter sp. Phosphobacteria and *Azospirillum* sp. (Combined) inoculated plants.

The morphological parameters like length of plant, number of leaves, breadth of leaves, length of leaves, shoot length, number of flowers, root length, total length of plants were increased in combined inoculation of FYM, *Rhizobium* sp., Azotobacter sp. Phosphobacteria and *Azospirillum* sp. *Cajanus cajan* L. plants than dual inoculations and control plants.

Bio-Chemical parameters like Chlorophyll content, Protein, Carbohydrate, Total free amino acids, Inorganic phosphorus, Reducing sugars, were also increased in combined treatment of FYM, *Rhizobium* sp., Phosphobacteria, Azotobacter sp. and *Azospirillum* sp. plants of *Cajanus cajan* L. than dual inoculation and control platens. This might be due to production of plant growth hormones and other plant growth substance.

The bacterial biofertilizers of with *Rhizobium* sp., Phosphobacteria and *Azospirillum* sp. inoculated plants showed increase in the growth of *Lablab purpureus* L. when compared with control plants. All the parameters like morphological and bio-chemical parameters increased in dual inoculated plants and more in FYM, *Rhizobium* sp., Phosphobacteria and *Azospirillum* sp. (Combined) inoculated plants.

The present study was well correlated with the previous reports by Gaur and Agarwadi(1989). They studied the combined and dual inoculations of *A.brasilense* and *Pseudomonas striata* in sorghum plant which increase in root length, nitorgenase activity, dry matter, seed yield as compared to single inoculation of both organisms and control plants.

Combined inoculation of *Rhizobium* and Phosphobacteria(*Bacillus megaterium* and

Pseudomonas striata) for red gram, black gram, green gram and Bengal gram increased the grain yield for maximum grain recorded by combination of rhizobial strain with phosphobacteria with full dose of N and P in red gram (Kannian,1999).

Effect of bacterial biofertilizers on various parameters of *Lablab purpureus L.* plants

Effect on length of plant

In *Lablab purpureus L.* the length of plants were increased in combined inoculations of FYM, *Rhizobium* sp., Phosphobacteria and *Azospirillum* sp. treated plants. The length of plants was recorded at 85.0 cm(combined inoculatiuons) followed by 80.9 in dual *(Rhizobium* sp., *Azospirillum* sp. and Phosphobacteria and 68.1 in control plants (Table.1; Figure. 4)

Effect on number of leaves

The number of leaves of plants treated with bacterial biofertilizers of combined inoculation recorded maximum followed by other inoculation. The observation on number of leaves of c *Lablab purpureus L.* treated with combined bioferilizers, dual, alone and control treatments were 24.0, 22.0 (*Rhizobium* sp. Azospirillum sp.and Phosphobacteria), 7.6 (*Rhizobium* sp.) and 6.8 respectively (Table.2 and Figure. 1).

Effect on breadth of leaves

The breadth of leaves was increased in *Lablab purpureus L.* plants in combined than dual, alone and control treatments. The observation on breadth of leaves of *Lablab purpureus L.* were 6.8, and 4.6 in control plants (Table.1 ;Figure. 3).

Effect on length of leaves

The length of leaves were increased in *Lablab purpureus L.* inoculated with *Rhizobium* sp., Phosphobacteria and *Azospirillum* sp. than dual and alone treatments. The observation of *Lablab purpureus L.* plants with combined, dual, alone and control were 9.2, 8.1 (*Rhizobium* sp. and *Azospirillum* sp.), 6.4 (Phosphobacteria) and 3.6 respectively (Table. 1; Figure. 2). Shukla and Gupta (1964) reported that the increase in length of leaves in rice plants treated with *P.foveloarum*.

Effect on shoot length

The observation on shoot length of *Lablab purpureus L.* inoculated with *Rhizobium* sp., Phosphobacteria and *Azospirillum* sp. (Combined), dual, alone and control were 79.4, 76.9 (*Rhizobium* sp. and *Azospirillum* sp.), 63.4 (*Azospirillum* sp) and 55.0 respectively (Table. 1; Figure. 5). Preeti Vasudevan *et al*.,(2002) studied that the increase in shoot length in rice plants treated with biological preparations(*Bacillus* sp.) when compared with control plants.

Effect on number of flowers

The number of flowers of *Lablab purpureus L.* plants inoculated with *Rhizobium* sp., Phosphobacteria and *Azospirillum* sp. were recorded maximum than dual and control plants. The observation on number of flowers of *Lablab purpureus L.* inoculated at combined treatments were 11.0 followed by 10.0 in dual (*Rhizobium* sp.and *Azospirillum* sp.), 8.0 in alone (Phosphobacteria and Rhizobium sp.). (Table. 2; Figure. 9).

Effect on root length

Root length of *Lablab purpureus L.* were increased in combined inoculation of bacterial biofertilizers were 36.9, 30.3 in dual (*Rhizobium* sp. and Phosphobacteria) and 19.3 in alone (Phosphobacteria sp.) treatments (Table.2; Figure. 6). This was correlated with pervious report by Preeti Vasudean *et al*.,(2002). They reported that the increase length of root when compared tot he control plants on CV.IR24 with four biological preparations(*Bacillus* sp.) on IR50 and Jyothi with five biological preparations of *Bacillus* sp.

Effect on nodulation

The observation on number of nodules of *Lablab purpureus L.* inoculated with combined biofertilizers were recoreded maximum than other treatments. The number of nodules were 33.0, 25.0(*Rhizobium* sp. and Phosphobacteria sp.) and 12.2 (*Rhizobium* sp.) respectively (Table. 2; Figure. 8). This is well accepted with previous reports by Saxena and Tilak(1999). They studied the seeds of pulse variety treated with *Rhizobium* which increase the yield through for better nodulation and maintain of organic matter in soil.

Effect on total length of plants

In *Lablab purpureus L.* the total lengths of plants were increased in combined inoculation of biofertilizers than other treatments. Their observations were 122.9, 110.3 in dual (*Rhizobium* sp. and Phosphobacteria) and 101.1 in alone (*Rhizobium* sp.) treatments (Table. 2; Figure. 7).

Effect on bio-chemical parameters

Effect on chlorophyll content

Then cholophyll content of *Lablab purpureus L.* gram plants inoculated with *Rhizobium* sp., Phosphobacteria and *Azospirillum* sp. were recorded maximum followed by dual, alone and control plants. In *Lablab purpureus L.* the chrolophyll content was increased in combined inoculation of *Rhizobium* sp., Phosphobacteria and *Azospirillum* sp. treatments were 5.89mg/g than in control plants(Table. 3. Figure. 10).

Effect on protein content

The protein content of *Lablab purpureus L.* inoculated with combined treatments of *Rhizobium* sp., Phosphobacteria and *Azospirillum* sp. were recorded maximum followed by dual, alone and control plants. The protein content of *Lablab purpureus L.* plants were 4.36 mg/g, 1.17(*Rhizobium* sp and Phosphobacteria), and 0.25 in control plants .(Table. 3; Figure. 11).

Effect on carbohydrate

The combined inoculation of *Rhizobium* sp., Phosphobacteria and *Azospirillum* sp. treated plants of *Lablab purpureus L.* were recorded maximum followed by dual, alone and control plants . The cabohydrate contents of *Lablab purpureus L.* were 23.80 mg/g, 21.57 (*Rhizobium* sp and *Azospirillum* sp.),14.80(*Rhizobium* sp.) and 11.0 respectively on 40 DAS (Table. 3; Figure. 12).

Effect on total free amino acids

The total free acids of *Lablab purpureus L.* plants treated with *Rhizobium* sp., Phosphobacteria and *Azospirillum* sp. were showed maximum than dual, alone and control plants. The total free amino acids contents of *Lablab purpureus L.* plants were 18.46 mg/g. 15.75 (*Rhizobium* sp and Phosphobacteria), 7.10 (*Rhizobium* sp.) and 2.25 respectively on 40 DAS (Table. 4; Figure. 13).

Effect on reducing sugar

The reducing sugar content on *Lablab purpureus L.* with combined treatments of *Rhizobium* sp., Phosphobacteria and *Azospirillum* sp. was found to be 5.43 mg/100g, 4.95 in dual (Phosphobacteria and *Azospirillum* sp.), 3.40 in alone (*Azospirillum* sp.) and 18.0 in control plants (Table. 4; Figure. 14).

Effect on inorganic phosphorus content

Rhizobium sp., Phosphobacteria and *Azospirillum* sp. combined treatments of *Lablab purpureus L.* plants, the inorganic phosphorus contents were showed maximum than dual, alone and control plants. The increase in inorganic content was observed in *Lablab purpureus L.* plants of combined treatments were 6.17 mg/g, 5.90 (*Rhizobium* sp and Phosphobacteria)on 40 DAS (Table. 4 ;Figure. 15).

Preeti Vasudevan *et al*., (2002) studied that the increase in shoot length in rice plants treated with biological preparations(*Bacillus* sp.) when compared with control plants. The present study was well correlated with the previous reports by Gaur and Agarwadi(1989). They studied the combined and dual inoculations of *A.brasilense* and *Pseudomonas striata* in sorghum plant which increase in root length, nitorgenase activity, dry

Table.1 Biochemical tests for identification

S.No	Biochemical tests	Rhizobium	Azotobacter sp.	Azospirillum sp.	Phophobacteria sp.
1	Gram Staining	G- ve	G- ve	G+ ve	G- ve
2	Motility test	Motile	Motile	Motile	Motile
3	Catalase	+ve	+ve	+ve	+ve
4	Oxidase	+ve	+ve	+ve	+ve
5	Indole	+ve	+ve	-ve	-ve
6	MR	+ve	+ve	+ve	+ve
7	VP	-ve	-ve	-ve	-ve
8	Citrate	+ve	+ve	+ve	+ve
9	TSI	+ve	+ve	+ve	+ve
10.	Sugar fermentation tests				
	Lactose	-ve	-ve	+ve	-ve
	Maltose	-ve	+ve	+ve	+ve
	Glucose	+ve	+ve	+ve	+ve
	Fructose	+ve	+ve	+ve	-ve
	Sucrose	+ve	+ve	+ve	+ve
	Mannitol	+ve	+ve	+ve	+ve
	Cellulose	-ve	+ve	+ve	+ve
	Galactose	+ve	+ve	+ve	+ve
11	N2 Reduction test	-ve	+ve	+ve	+ve
12	H2S Production	-ve	+ve	+ve	+ve
13	Starch hydrolysis	+ve	+ve	+ve	+ve

Table.1 Effect of morphological parameters of *Lablab purpureus* L. inoculated with bacterial biofertilizers

Treatments	**Number of leaves/plant**	**Length of leaves (cm)**	**Breadth of leaves (cm)**	**Length of plant (cm)**	**Shoot length (cm)**
Control (C)	3.4	5.8	1.0	17.9	11.6
Rhizobium sp. (T1)	5.0	6.3	1.2	28.5	15.8
Azospirillum sp (T2)	4.4	6.42	1.5	26.2	14.7
Phosphobacteria sp. (T3)	5.4	7.0	1.5	26.2	15.3
Azotobacter sp. (T4)	5.8	6.9	1.48	29.4	16.9
Rhizobium sp. +*Azospirillum* (T5)	5.0	6.5	1.44	28.5	16.3
Phophobacteria + *Azospirillum* sp *(T6)*	5.2	6.4	1.3	27.7	15.2
Rhizobium sp. + *Azotobacter* sp. *(T7)*	5.4	6.7	1.26	30.7	15.4
Azotobacter sp.+ *Azospirillum* sp. (T8)	6.0	7.2	1.4	30.0	15.6
Rhizobium +Phophobacteria +*Azospirillum*+ *Azotobacter* sp. (T9)	6.6	7.4	1.5	33.5	17.0

Table.2 Effect on yield concepts of *Lablab purpureus* L. inoculated with bacterial biofertilizers

Treatments	Number of nodules/plant	Number of flowers/ plant	Root length (cm)
Control (C)	0.0	1.8	4.4
Rhizobium sp. (T1)	10.0	3.2	7.6
Azospirillum sp (T2)	2.0	3.8	6.8
Phosphobacteria sp. (T3)	2.0	3.6	7.2
Azotobacter sp. (T4)	5.4	3.2	8.1
Rhizobium sp. +*Azospirillum* (T5)	9.0	2.8	7.4
Phophobacteria + *Azospirillum* sp *(T6)*	3.0	2.6	8.8
Rhizobium sp. + *Azotobacter* sp. *(T7)*	3.4	4.6	8.8
Azotobacter sp.+ *Azospirillum* sp. (T8)	0.0	3.2	7.7
Rhizobium +Phophobacteria +*Azospirillum*+ *Azotobacter* sp. (T9)	13.6	4.8	9.6

Table.3 Effect of biochemical parameters of *Cajanus cajan* L. inoculated with bacterial biofertilizers

Treatments	Chlorophyll (mg/g)	Protein (mg/g)	Carbohydrate (mg/g)
Control (C)	0.70	0.25	11.0
Rhizobium sp. (T1)	1.06	0.30	14.01
Azospirillum sp (T2)	1.37	0.27	14.80
Phosphobacteria sp. (T3)	1.57	0.33	14.80
Azotobacter sp. (T4)	1.62	0.62	15.11
Rhizobium sp. +*Azospirillum* (T5)	1.91	0.54	15.27
Phophobacteria + *Azospirillum* sp *(T6)*	1.99	0.56	15.51
Rhizobium sp. + *Azotobacter* sp. *(T7)*	5.21	4.17	21.57
Azotobacter sp.+ *Azospirillum* sp. (T8)	3.01	1.07	16.78
Rhizobium +Phophobacteria +*Azospirillum*+ *Azotobacter* sp. (T9)	2.80	0.72	12.94

Table.4 Effect of biochemical parameters of *Cajanus cajan* L. plants inoculated with bacterial biofertilizers

Treatments	Reducing sugar (mg/g)	Amino acids (mg/g)	Inorganic phosphorus (mg/g)
Control (C)	1.80	2.25	2.08
Rhizobium sp. (T1)	1.90	5.10	2.26
Azospirillum sp (T2)	3.40	5.60	2.13
Phosphobacteria sp. (T3)	1.90	7.60	2.26
Azotobacter sp. (T4)	3.80	9.69	2.58
Rhizobium sp. +*Azospirillum* (T5)	3.30	9.18	2.45
Phophobacteria + *Azospirillum* sp *(T6)*	3.70	11.73	2.64
Rhizobium sp. + *Azotobacter* sp. *(T7)*	4.95	15.75	5.90
Azotobacter sp.+ *Azospirillum* sp. (T8)	3.36	11.68	3.40
Rhizobium +Phophobacteria +*Azospirillum*+ *Azotobacter* sp. (T9)	65.43	8.17	2.93

Figure 1. Effect of Length of leaves *Lablab purpureus* L. inoculated with bacterial biofertilizers

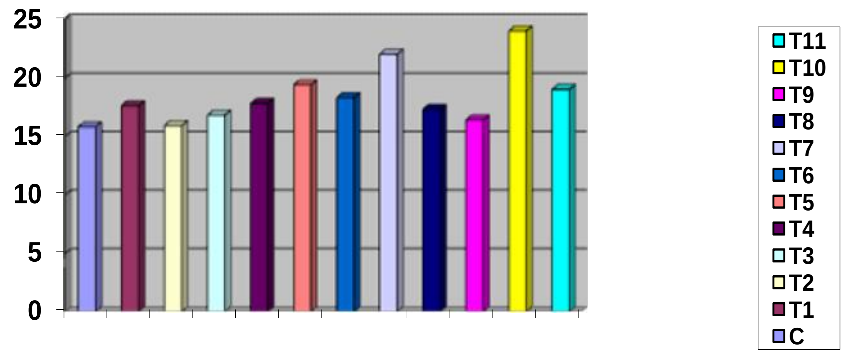


Figure 2. Effect of Number of leaves *Lablab purpureus* L. inoculated with bacterial biofertilizers

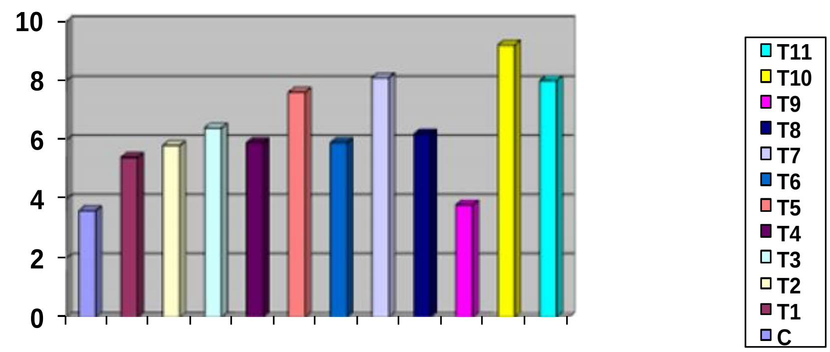


Figure 3. Effect of Breadth of leaves *Lablab purpureus* L. inoculated with bacterial biofertilizers

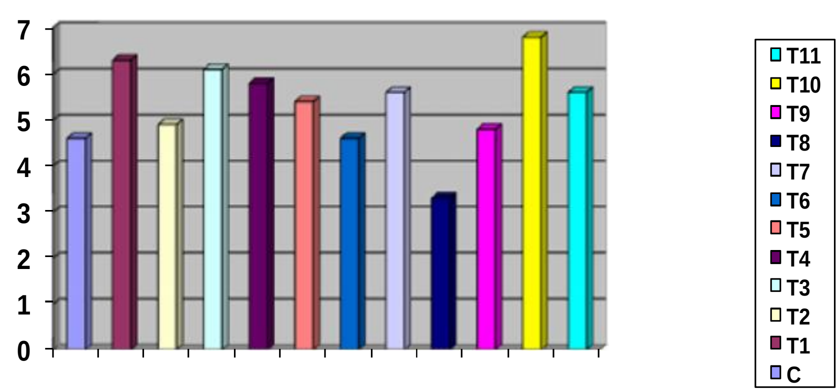


Figure 4. Effect of Length of Plants *Lablab purpureus* L. inoculated with bacterial biofertilizers

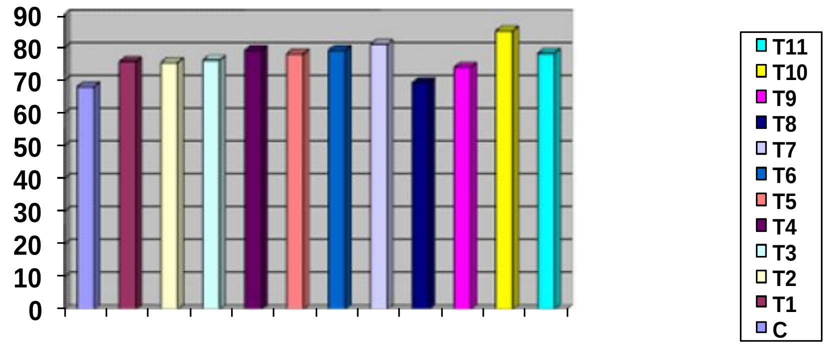


Figure 5 . Effect of Shoot length *Lablab purpureus* L. inoculated with bacterial biofertilizers

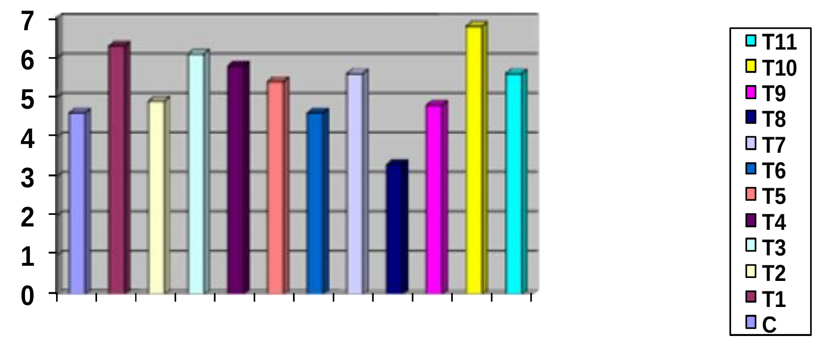


Figure 6. Effect of Root length *Lablab purpureus* L. inoculated with bacterial biofertilizers

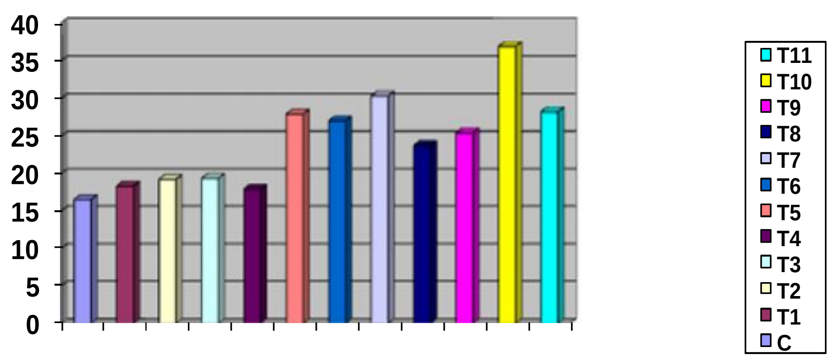


Figure 7. Effect of Total length of plants *Lablab purpureus* L. inoculated with bacterial biofertilizers

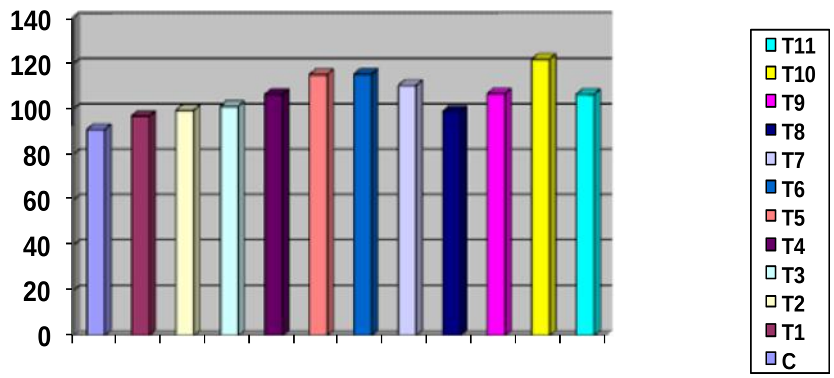


Figure 8. Effect of Reducing sugars *Lablab purpureus* L. inoculated with bacterial biofertilizers

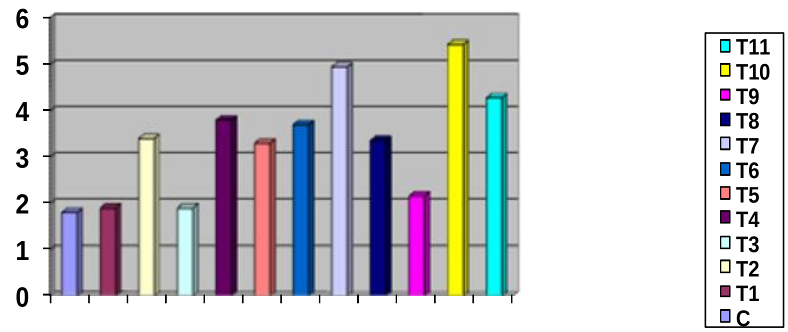


Figure 9. Effect of No. of flowers *Lablab purpureus* L. inoculated with bacterial biofertilizers

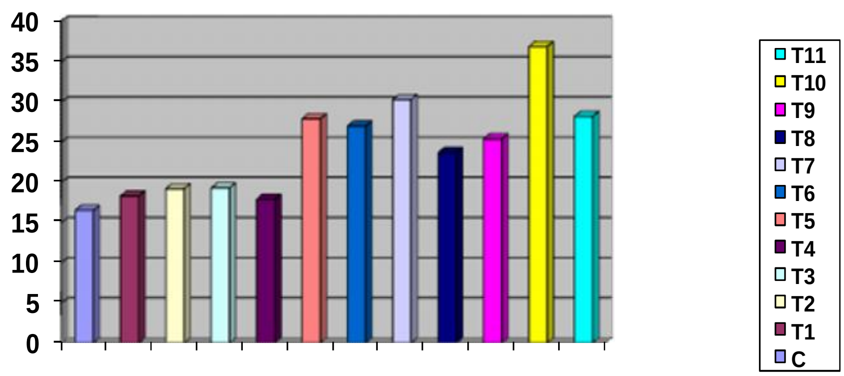


Figure 10. Effect of Chlorophyll content of plants *Lablab purpureus* L. inoculated with bacterial biofertilizers

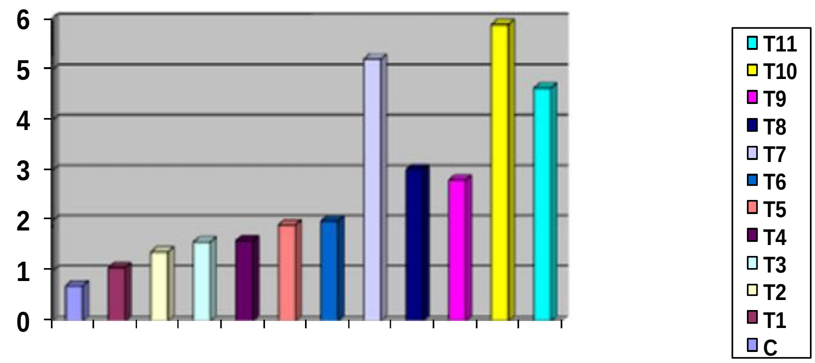


Figure 11. Effect of Protein *Lablab purpureus* L. inoculated with bacterial biofertilizers

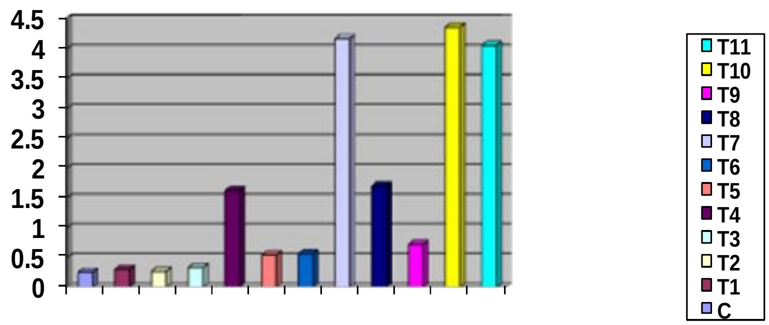


Figure 12. Effect of Carbohydarate content of plants *Lablab purpureus* L. inoculated with bacterial biofertilizers

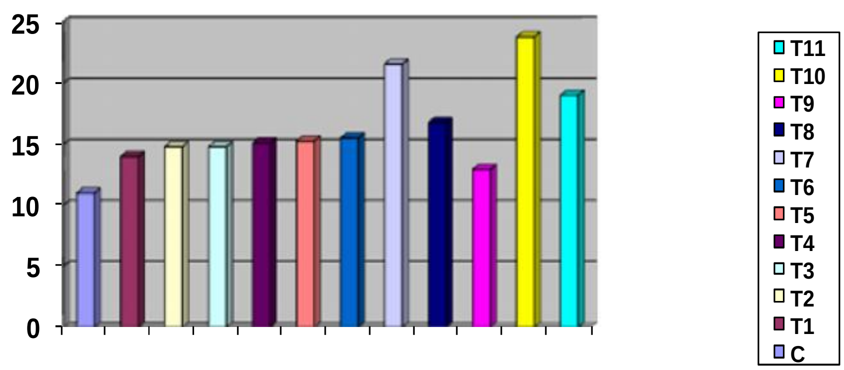


Figure 13. Effect of total free amino acids *Lablab purpureus* L. inoculated with bacterial biofertilizers

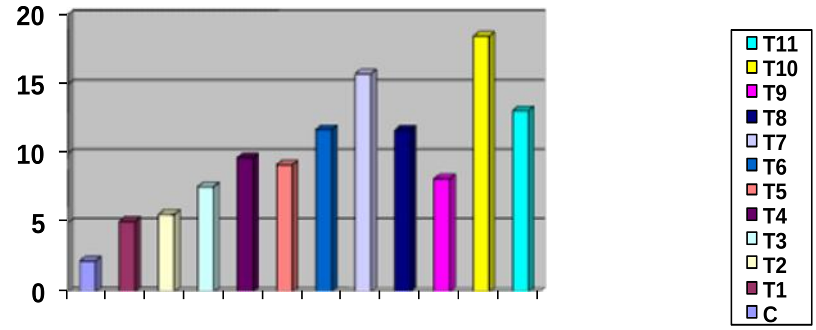


Figure 14. Effect of inorganic phosphorus content of plants *Lablab purpureus* L. inoculated with bacterial biofertilizers

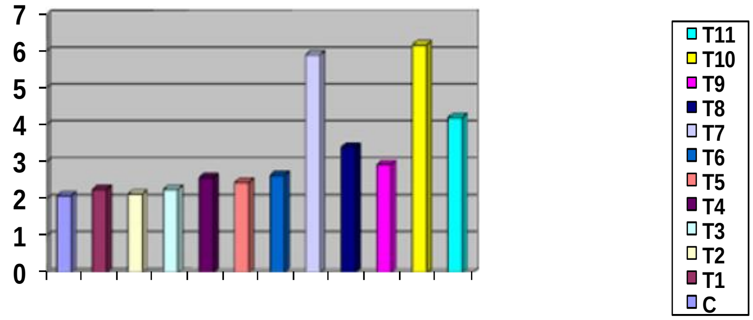
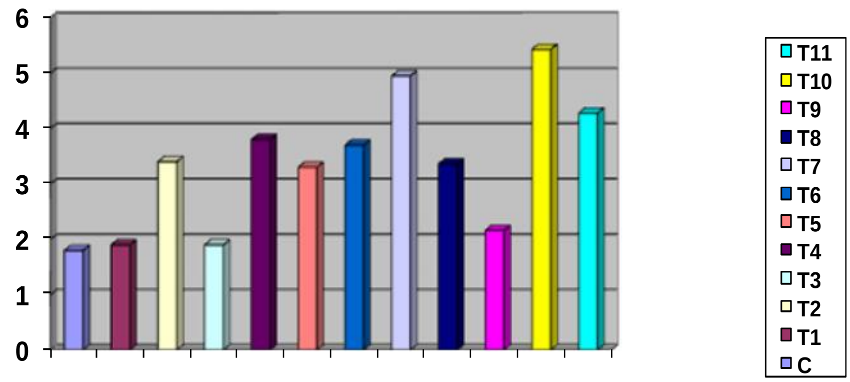


Figure 15. Effect of Reducing sugars *Lablab purpureus* L. inoculated with bacterial biofertilizers



matter, seed yield as compared to single inoculation of both organisms and control plants. Combined inoculation of *Rhizobium* and Phosphobacteria(*Bacillus megaterium* and *Pseudomonas striata*) for red gram, black gram, green gram and Bengal gram increased the grain yield for maximum grain recorded by combination of rhizobial strain with phosphobacteria with full dose of N and P in red gram (Kannian,1999).

From the experiments, it is clearly proved that applying bacterial biofertilizers considerably improve the growth and yield of *Cajanus cajan* L. plants, Hence, it could reduces the dose of other chemical fertilizer used, which cause pollution to the environment, it helps the economically poor farmers.

References

Dwivedi, R.S; Dubey, R.C and Dwivedi, S.K. 1989. In: Plant – Microbe Interactions, Ed. Bilgrame, K.S focal Them Botany ISCA symposium, Narendra Publ. House, Delhi, 217-238.

Glick, B.R. and Bashan, Y. 1997. *Biotechnol. Adv*., **15**: 353-378.

Gnanamanickam, S.S., Vasudevan, P., Reddy, M.S., Klopper, J.W. and Defago, G., 2002. In: Biological control of Crop Disease, Marcel Dekker, New York. Pp. 1 – 9.

Kannaiyan, S. 1999. Bioresources Technology for Sustainable Agriculture, Associated Publishing Company, New Delhi. P.422.

Kloepper, J.W., Lifshitz, R, and Zablotowicz, R.M., 1989. *Ibid*, **7:** 39 – 43.

Kloepper, J.W.Leong, J., Teintze, M. and Schroth, M.N. 1980. *Nature*, 286 : 885 – 886.

Lambert, B. and Joos, H., 1989. *Trends Biotechnol*., 7: 215 – 219.

Preeti Vasudevan, Reedy, M.S Kavitha. S, Vellusamy.P., David Paulraj. R.S. Purosothaman. S.M. Brinda Priyadarisini. V, Bharathkumar. S, Kloepper.J.W, and Gnanamanickam. S.S. 2002. Role of biological preparations in enhancement of rice seedling growth and grain yield. *Curr. Sci*., 83 9 : 1140 – 1143.

Rewari, R.B. 1984 & 1985. Summarized result of Microbiology Trials. All India coordinated Research project on Improvement of pulses, ICAR, New Delhi.

Subba Rao, N.S. 1982. Biofertilizers, In: Advances in Agricultural Microbiology Ed. Subba Rao. N.S.. Oxford and IBH Publ. Co., New Delhi. Pp 219 – 242.

Subba Rao, N.S. 2002. Soil Microbiology. Oxford and IBH publ co. ltd. New Delhi.

Subba Rao, N.S. and Singh, C.S. 1985. *Zent. Microbial*. 140: 97 – 102.

Subba Rao, N.S. and Tilak, K.B.R. 1977. Souvenir Bull. Directorate of Pulse Development, Govt. of India.

Tang, W.H. 1994. In: Improving Plant Productivity with Rhizosphere Bacteria Eds Reder, M.H. Stephens, P.M. and Bowen, G.D.. CSIRO, Adelaide. Pp. 267 – 278.

Venkataraman, G.S. 1972. Algal Biofertilizers and Rice cultivation, today and Tomorrow Printers and Publ. New Delhi. Pp. 75.

.