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Research Article

# Effect of bacterial biofertilizers on the growth and yield of *Phaseolus vulgaris* L.

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**Abstract**

The present investigation was carried out to study the effect of bacterial Biofertilizers on pulse crop like *Phaseolus vulgaris* L. Bacterial biofertilizers like *Rhizobium* sp., Phosphobacteria, *Azotobacter* sp and *Azospirillm* sp. were isolated from the soils of agricultural crops by employing plating techniques. The isolation was done by selective medium such as *Rhizobium* medium and Yeast Extract Mannitol Agar for *Rhizobium* sp., Pikovskaya's agar for Phosphate solubilizing microbes and Semisolid agar for *Azospirillum* sp,Ashbys mannitol agar for *Azotobacter* sp. The isolated bacterial members were identified by Gram's staining, motility and sugar fermentation methods. These bacterial members were used as inoculants for seed treatments. Seeds of *Phaseolus vulgaris*.L were treated with bacterial biofertilizers, the treatment like Phosphobacteria, *Azospirillum* sp., *Rhizobium* sp. (Alone inoculation), Phosphobacteria and *Azospirillum* sp. *Azotobacter* sp *Rhizobium* sp. and *Azospirillum* sp., *Rhizobium* sp. and Phosphobacteria (Dual inoculation) and *Azotobacter* sp, *Rhizobium* sp., Phosphobacteria and *Azospirillum* sp. (Combined inoculation). The microbial inoculants were sowed in sterile polythene bags containing sterilized soil samples. Controls were also maintained without a bacterial biofertilizers. After 50 days of sowing, the plant growth parameters like morphological and bio-chemical parameters were analyzed in *Phaseolus vulgaris L.* The morphological parameters like length of plant, number of leaves, breadth of leaves, length of leaves, shoot length, number of flowers, root length,no of seeds,no of pods were increased in combined inoculation of *Azotobacter* sp, *Rhizobium* sp., Phosphobacteria and *Azospirillum* sp. *Phaseolus vulgaris L.* 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 *Azotobacter* sp, *Rhizobium* sp., Phosphobacteria and *Azospirillum* sp. plants of *Phaseolus vulgaris L.*. than dual inoculation and control plants. This might be due to production of plant growth hormones and other plant growth substance. From the experiments, it is clearly proved that applying bacterial biofertilizers considerably improve the growth and yield of *Phaseolus vulgaris L.* Hence, it could reduces the dose of other chemical fertilizer used, which cause pollution to the environment, it helps the economically poor farmers.

**Keywords:** Bacterial Biofertilizers, *Phaseolus vulgaris* L, plant growth parameters.

## Introduction

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).

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.

Pulses are second only to cereals in their important as human food especially in India, where the people derive most of their protein requirements from these cops. Since the average diet of the Indian population is much deficient in protein content, there is need for a several food increase in the production of pulses. In recent years much emphasis has been directed towards increased cultivation of pulse crops. Since, intensive cultivation practices often create new and more severe plant disease problem, it is essential to know the various disease of these corps and how to cope with them. Besides serving as valued human food. Pulse crops are valued for their Nitrogen fixing quality, in symbiotic relationship with the bacterium *Rhizobium* in their root nodules. They are commonly rotated with cereals and other crops in most areas of the country, in order to enrich the soil.

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 *Azotobacter* sp and *Azospirillum* sp. can also exploit to promote the growth and yield of pulse crop by using them as biofertilizers.

## Aim and objectives

- Effect of Bacterial biofertilizers (*Rhizobium* sp., Phosphobacteria *Azotobacter* sp and *Azospirillum* sp.) on different growth parameters of *Phaseolus vulgaris* 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.
- Estimation 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 *Phaseolus vulgaris* L.

Biofertilizers such as *Rhizobium* sp., Phosphobacteria *Azotobacter* sp and *Azospirillum* 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 of2:1 (w/v). The sand soil mixture was sterilized at 121ºC (151bs) for one nacre for two 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 fro 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. 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.

**Isolation of *Azotobacter* sp from soil sample**

From eacm dilution, 0.1ml of sample was taken and spread plates technique was perfomed on Ashby's mannitol agar. The plates were inocubated for 3-4 day Every 24 hours, the plates were checked for the hat of *Azotobacter* 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.

**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 *Phaseolus vulgaris L.* plants were added into the slurry. The seeds were sown in the Pot containing sterilized soil samples.

**Inoculation of bacterial biofertilizers in the soil**

Treatments were as follows
C - Control plants
T1 - seeds of ***phaseolus vulgaris*** *L.*. treated with Rhizobium sp.
T2 - seeds of *Phaseolus vulgaris L.*. **.** treated with *Azospirillum* sp
T3 - Seeds of *Phaseolus vulgaris*.L *Azotobacter* sp.
T4 - seeds of *Phaseolus vulgaris L.*. treated with *Phosphobacterium*
sp.
T5 - seeds of *Phaseolus vulgaris L.*. treated with Rhizoium and *Azospirillum* sp.
T6 - seeds of *Phaseolus vulgaris L.*. treated with *Rhizobium* sp. and *Phosphobacteria* sp.
T7 - seeds of *Phaseolus vulgaris L.*. treated with *Azotobacter* sp. and *Rhizobium* sp.
T8 - seeds of *Phaseolus vulgaris L.*. treated Urea and *Azospirillum* sp. and Phosphobacteria.
T9 - seeds of *Phaseolus vulgaris L.*. *Rhizobium* sp., *Azospirillum* sp and *Azotobacter* sp.
T10 - seeds of *Phaseolus vulgaris L.*. treated *Rhizobium* sp., Phosphobacteria and *Azospirillum* sp.
T11 - seeds of *Phaseolus vulgaris L.*. treated *Azotobacter* sp, *Rhizobium* sp., Phosphobacteria and *Azospirillum* sp.

After 50 days of sowing the morphological and bio-chemical parameters of *Phaseolus vulgaris* 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,o of nodules,no of seeds,no of pods, *Phaseolus vulgaris L.*. 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.

**Estimation of biological compounds**
**Estimation of chlorophyll content (Arnon,1949)**

1 gm of finely cut sample of leaves were taken and ground to a fine pulp with the addition of 20 ml acetone. Then it was centrifuged and the supernatant was transferred to a 100 ml volumetric flask. Then the residue was ground with 20 ml of acetone,centrifuged and the supernatant was transferred to the same volumetric flask. The volume was made up of 100 ml with 80% acetone.The absorbence were read at 645 and 663 nm against the solvent blank.

**Extraction of sample**

500 mg of the sample was weighed and ground with 10% TCA(5ml) using a morter and pestle . The ground sample was centrifuged and the residue containing sample was mixed with 0.1N NaOH (5ml ). The solution was again centrifuged at 2000 rpm for 10 minutes. The supernatant was collected for protein estimation.

**Estimation of protein**

For test 0.1 ml and 0.2 ml of diluted sample was taken and made up to 1 ml with distilled water. Add 5 ml of reagent C to each tube including blank and was allowed to stand for 10 minutes. Exactly 0.5 ml of diluted Folin's reagent was added to all tubes with continuous shaking and allowed to stand for 30 minutes. The colour developed was read at 645 nm using reagent blank. Then O.D value was obtained and compared with the standard graph which was plotted using BSA as standard and the concentration of unknown protein was calculated.

**Estimation of carbohydrate ( Hedge and Hofriter,1962)**

The sample (supernatant) were taken in a series of test tubes from 0.1 to 0.5 concentration and made up to 1 ml with distilled water. Then 4 ml of freshly prepared anthrone reagent was added into each test tubes. The test tubes were kept in boiling water path for 10 minutes. Then cooled rapidly and optical density was measured at 630 nm. From the standard, the unknown carbohydrate present in the sample was calculated.

**Estimation of total free amino acids ( Moore and Stein,1948)**

**Extraction of amino acids**

500 mg of sample was weighed and ground with small quantity of acid washed sand. 5 ml to 10 ml of 80% ethanol was added and then filtered through filter paper. The filtrate was collected. The residue was ground and centrifuged. After centrifugation the supernatant was collected. The extraction was repeated twice. The filtrate and supernatant were mixed and used for amino acid estimation.

**Estimation of amino acids:**

0.1 ml of supernatant was taken and made up to 1 ml with distilled water. 1 ml of ninhydrin reagent was added.

The test tubes were kept in boiling water path for 20 minutes. 5 ml of diluent was added and mixed well. After 15 minutes the absorbance was read at 570 nm. The O.D value obtained was compared with the standard concentration of amino acids.

**Estimation of reducing sugars (Somogyi, 1952)**

**Extraction**

One gram of fresh tissue was homogenized with 80% alcohol and extracted repeatedly with boiling 80% alcohol, until the last traces of sugar were removed. The alcoholic extracts were centrifuged and the supernatant was made up to a known volume.

**Method**

To 0.2 ml of the above solution 1 ml litre of reagent I was added, heated for 10 min in a vigorously boiling water bath and cooled. 1 ml of reagent II was added and the solution was diluted to 10ml with distilled water. Absorbance was read at 500 nm using a spectrophotometer. The reducing sugar content was estimated from a calibrated standard curve of D – glucose and expressed as mg glucose equivalent $g^{-1}$ fresh wt.

**Estimation of inorganic phosphorus**

**Extraction of samples**

3g of leaves were homogenized and dissolved in glacial acetic acid. It was made upto 100ml with sterile distilled water. From that, 1ml was taken, and diluted to 10ml with the same.

**Estimation of inorganic phosphorus**

0.1ml to 2.5ml of working standard was pipetted out in distilled test tubes. 1ml of sample was taken in the test tubes. The volume of test tubes was made upto 7ml with distilled water. 1ml of molybdenum solution was added to all the tubes followed by 0.4ml of Amino Napthol Sulphonic acid reagent. The test tubes allowed to strand for 20 minutes. The intensity of the colour developed was read at 680nm against blank
From the standard, the concentration of inorganic phosphorus present in the samples was calculated.

## Results and Discussion

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

**Isolation of bacteria**
**Isolation of *Rhizobium* sp. from the root nodules**
**Colony morphology**

*Rhizobium* sp. colonies are white translucent, glistening, elevated, small colonies with margin mucoid colonies.

**Gram reaction**
Gram negative rods
**Motility test**
Motile

**Bio-chemical tests**
*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 A*zospirilum*** sp.

**Colony morphology**
*Azospirillum* sp. colonies are white pellicles,2-4mm below the surface of the medium., glistening, elevated, small colonies with margin mucoid colonies
**Gram reaction**
Gram negative rods
**Motility test**
Motile
**Bio-chemical tests**
Ferments glucose, fructose and sucrose.

**Isolation of Azotobacter sp**

**Colony morphology**
Azotobacter sp.colonieswhite translucent, glistening, elevated, mucoid, small colonies with margin mucoid colonies
**Gram reaction**
Gram negative rods
**Motility test**
Motile
**Bio-chemical test**
Azotobacter sp. ferments glucose, lactose and sucrose
On Ashby mannitol agar, Azotobacter colonies produce gum like substances and appeared as mucoid colonies. These substansces are made up water soluble extracellular polysaccharides.

**Isolation of phosphobacteria**

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

**Field experiment**

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

**Effect on length of plant**

In *Phaseolus vulgaris L.* the length of plants were increased in combined inoculations of *Rhizobium* sp., Phosphobacteria,*Azotobacte* sp and *Azospirillum* sp. treated plants. The length of plants was recorded at 27.6 cm(combined inoculatiuons) followed by 22.6 in dual *(Rhizobium* sp., *Azospirillum* sp. and Phosphobacteria and 15.0 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 *Phaseolus vulgaris L.* treated with combined bioferilizers, dual, alone and control treatments were 15.0, 15.0 (*Rhizobium* sp. Azospirillum sp.and Phosphobacteria), 7.0 (*Rhizobium* sp.) and 6.8 respectively (Table.2 and Figure. 1).

**Effect on breadth of leaves**

The breadth of leaves was increased in *Phaseolus vulgaris L.* plants in combined than dual, alone and control treatments. The observation on breadth of leaves of *Phaseolus vulgaris L.* were 4.7, and 1.9 in control plants (Table.1:Figure. 3).

**Effect on length of leaves**

The length of leaves were increased in *Phaseolus vulgaris L.* inoculated with *Rhizobium* sp., Phosphobacteria and *Azospirillum* sp. than dual and alone treatments.

The observation of *Phaseolus vulgaris L.* plants with combined, dual, alone and control were 8.6, 8.4 (*Rhizobium* sp. and *Azospirillum* sp.), 5.2(*Rhizobium* sp) and 5.2 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 *Phaseolus vulgaris L.* inoculated with *Rhizobium* sp., Phosphobacteria,*Azotobacter* sp and *Azospirillum* sp. (Combined), dual, alone and control were 17.2, 16.2 (*Rhizobium* sp. And *Rhizobium* sp, phosphobacteria.), 10.7 (*Azospirillum* sp) and 8.4respectively (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 *Phaseolus vulgaris L.* plants inoculated with *Rhizobium* sp., Phosphobacteria *Azotobacter* sp and *Azospirillum* sp. were recorded maximum than dual and control plants. The observation on number of flowers of *Phaseolus vulgaris L.* inoculated at combined treatments were 12.0 followed by 5.0 in dual ( *Rhizobium* sp, ,*Azobacter* sp.and *Azospirillum* sp.), 8.0 in alone (*Azospirillum* sp ). (Table. 2; Figure. 9).

**Effect on root length**

Root length of *Phaseolus vulgaris L.* were increased in combined inoculation of bacterial biofertilizers were 10.8, 7.6 in dual (*Rhizobium* sp, *Azospirillum* sp and *Azotobacter* sp ) and 6.8 in alone (*Azotobacter* 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 *Phaseolus vulgaris L.* inoculated with combined biofertilizers were recoreded maximum than other treatments. The number of nodules were 13.0, 12.0(*Rhizobium* sp,*Azotobacter* sp and *Azospirillum* sp.) and 4.0 (*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 seeds**

In *Phaseolus vulgaris L.* the seeds of plants were increased in combined inoculation of biofertilizers than other treatments. Their observations were 12.0, 8,0 in dual (*Rhizobium* sp, *Azotobacter* sp. and Phosphobacteria) and 8.0 in alone (*Rhizobium* sp.) treatments (Table. 2; Figure. 7).

**Effect on pods**

In phaseolus vulgaris L.the pods of plants were increase in combind inoculation of biofertilizers then

other treatments. Their observations were 11.0,5.0(*Rhizobium* sp,*Azospirillum sp and Azotobacter sp*) and 8.0 in alone (*Rhizobium* sp) treatments.

**Effect on bio-chemical parameters**

**Effect on chlorophyll content**

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

**Effect on protein content**

The protein content of *Phaseolus vulgaris L.* inoculated with combined treatments of *Rhizobium* sp., Phosphobacteria, *Azotobacter* sp and *Azospirillum* sp. were recorded maximum followed by dual, alone and control plants. The protein content of *Phaseolus vulgaris L.* plants were 12.36 mg/g, 4.17(*Rhizobium* sp, Azotobacter sp and Phosphobacteria), and 0.25 in control plants .(Table. 3; Figure. 11).

**Effect on carbohydrate**

The combined inoculation of *Rhizobium* sp., Phosphobacteria, *Azotobacter* sp and *Azospirillum* sp. treated plants of *Phaseolus vulgaris L.* were recorded maximum followed by dual, alone and control plants . The cabohydrate contents of *Phaseolus vulgaris L.* were 23.80 mg/g, 21.57 (*Rhizobium* sp, *Rhizobium* sp and *Azospirillum* sp.),14.80(*Rhizobium* sp.) and 11.0 respectively on 50 DAS (Table. 3; Figure. 12).

**Table.1 Effect of morphological parameters of *Phaseolus vulgaris* L. inoculated with bacterial biofertilizers**

| Treatments | Number of leaves | No of flowers (cm) | Plant length (cm) | Length of leaves (cm) | Breath of leaves (cm) |
|---|---|---|---|---|---|
| *Control* | 5 | 2 | 15 | 5.2 | 9.6 |
| *Rhizobium sp.* | 7 | 12 | 21.2 | 5.2 | 3.1 |
| *Azospirillum sp.* | 6 | 8 | 17 | 4.9 | 3.2 |
| *Azotobacter sp* | 6 | 10 | 15.6 | 7.7 | 3.8 |
| *Phosphobacteria sp* | 6 | 11 | 19.8 | 6 | 3.4 |
| *Rhizobium sp+Azospirillum* | 13 | 11 | 22.2 | 6.5 | 4.2 |
| *Arhizobium sp+ .+Phophobacteria* | 15 | 14 | 26.4 | 7.5 | 4.1 |
| *Rhizobium sp+Azotobacter* | 9 | 8 | 23.2 | 4.1 | 3.2 |
| *Phosphobacteria+Azospiri llum sp* | 9 | 5 | 21.4 | 4.8 | 3.3 |
| *Rhizobium sp+Azospirillum sp+Azotobacter sp* | 9 | 5 | 22.6 | 4.7 | 3.6 |
| *Rhizobium +Phophobacteria +Azospirillum* | 15 | 12 | 27.6 | 8.6 | 4.2 |
| *Rhizobium sp+Azotobacter+ sp+Phosphobacter +Azospirillum* | 15 | 13 | 17.4 | 8.5 | 5.1 |

**Table.2** Effect of yield concepts of *Phaseolus vulgaris* L. inoculated with bacterial biofertilizers

| Treatments | Root length (cm) | Stem length (cm) | No of seeds | No of pods | No of nodules |
|---|---|---|---|---|---|
| *Control* | 5.6 | 8.4 | 5 | 6 | 10 |
| *Rhizobium sp.* | 7.4 | 10.7 | 8 | 8 | 4 |
| *Azospirillum sp.* | 6.8 | 15.2 | 9 | 8 | 3 |
| *Azotobacter sp* | 6.8 | 11.2 | 7 | 8 | 4 |
| *Phosphobacteria sp* | 6.6 | 13.2 | 9 | 8 | 1 |
| *Rhizobium sp+Azospirillum* | 6.6 | 11.8 | 11 | 5 | 5 |
| *Arhizobium sp+ .+Phophobacteria* | 9.4 | 14.8 | 11 | 9 | 2 |
| *Rhizobium sp+Azotobacter* | 8.4 | 10.6 | 7 | 7 | 1 |
| *Phosphobacteria+Azospirillum sp* | 7.2 | 8 | 6 | 5 | 2 |
| *Rhizobium sp+Azospirillum sp+Azotobacter sp* | 7.6 | 7.4 | 8 | 5 | 12 |
| *Rhizobium +Phophobacteria +Azospirillum* | 10.2 | 17.2 | 12 | 11 | 13 |
| *Rhizobium sp+Azotobacter+ sp+Phosphobacter +Azospirillum* | 10.2 | 16.2 | 11 | 11 | 12 |

**Table. 3** Effect of biochemical parameters of *Phaseolus vulgaris* L. inoculated with bacterial biofertilizers

| Treatments | Chlorophyll (mg/g) | Protein (mg/g) | Carbohydrate (mg/g) |
|---|---|---|---|
| *Control* | 0.70 | 0.25 | 11.0 |
| *Rhizobium sp.* | 1.06 | 0.30 | 14.01 |
| *Azospirillum sp.* | 1.37 | 0.27 | 14.80 |
| *Azotobacter sp* | 1.57 | 0.33 | 14.80 |
| *Phosphobacteria sp* | 1.62 | 0.62 | 15.11 |
| *Rhizobium sp+Azospirillum* | 1.91 | 0.54 | 15.27 |
| *Arhizobium sp+ .+Phophobacteria* | 1.99 | 0.56 | 15.51 |
| *Rhizobium sp+Azotobacter* | 5.21 | 4.17 | 21.57 |
| *Phosphobacteria+Azospirillum sp* | 3.01 | 1.07 | 16.78 |
| *Rhizobium sp+Azospirillum sp+Azotobacter sp* | 2.80 | 0.72 | 12.94 |
| *Rhizobium +Phophobacteria +Azospirillum* | 5.89 | 12.36 | 19.02 |
| *Rhizobium sp+Azotobacter+ sp+Phosphobacter +Azospirillum* | 4.63 | 4.06 | 23.80 |

**Table. 4** Effect of biochemical parameters of *Phaseolus vulgaris* L. inoculated with bacterial biofertilizers

| Treatments | Reducing sugar (mg/g) | Amino acids (mg/g) | Inorganic phosphorus (mg/g) |
|---|---|---|---|
| *Control* | 1.80 | 2.25 | 2.08 |
| *Rhizobium sp.* | 1.90 | 5.10 | 2.26 |
| *Azospirillum sp.* | 3.40 | 5.60 | 2.13 |
| *Azotobacter sp* | 1.90 | 7.60 | 2.26 |
| *Phosphobacteria sp* | 3.80 | 9.69 | 2.58 |
| *Rhizobium sp+Azospirillum* | 3.30 | 9.18 | 2.45 |
| *Arhizobium sp+ .+Phophobacteria* | 3.70 | 11.73 | 2.64 |
| *Rhizobium sp+Azotobacter* | 4.95 | 15.75 | 5.90 |
| *Phosphobacteria+Azospirillum sp* | 3.36 | 11.68 | 3.40 |
| *Rhizobium sp+Azospirillum sp+Azotobacter sp* | 2.16 | 8.17 | 2.93 |
| *Rhizobium +Phophobacteria +Azospirillum* | 5.43 | 18.46 | 4.20 |
| *Rhizobium sp+Azotobacter+ sp+Phosphobacter +Azospirillum* | 4.28 | 13.08 | 6.17 |

**Figure 1 .** Effect of Length of leaves *Phaseolus vulgaris* L. inoculated with bacterial biofertilizers

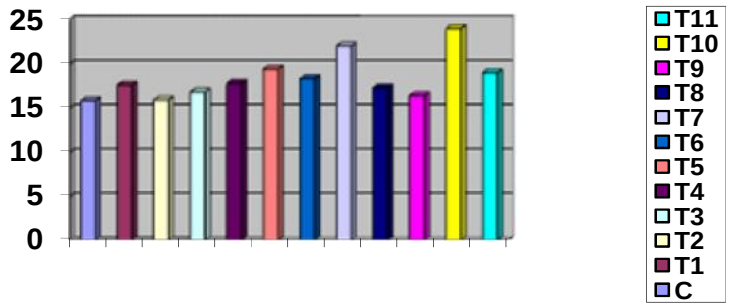



**Figure 2..** Effect of Number of leaves *Phaseolus vulgaris* L. inoculated with bacterial biofertilizers

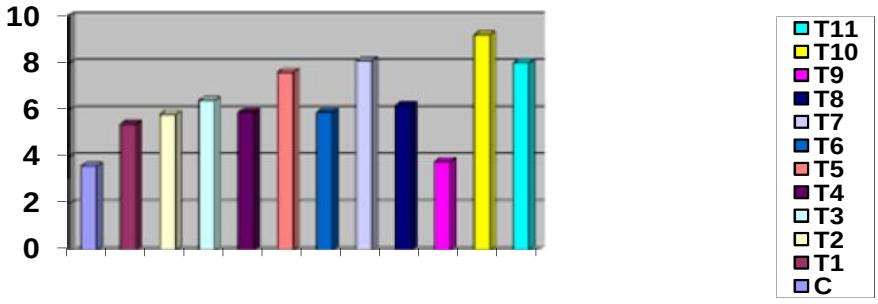


**Figure 3 .** Effect of Breadth of leaves *Phaseolus vulgaris* L. inoculated with bacterial biofertilizers

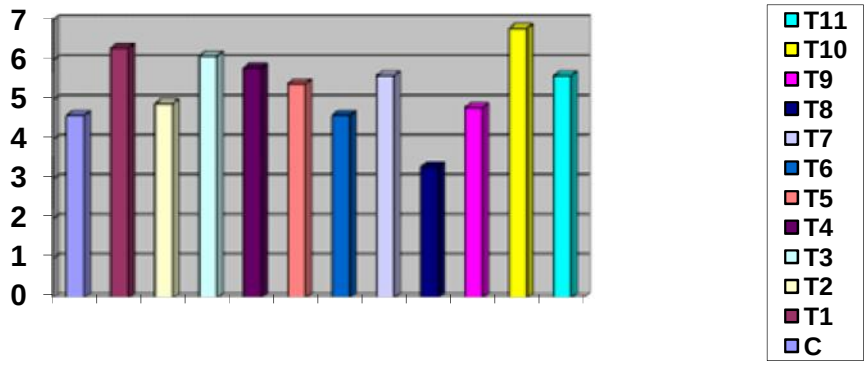



**Figure 4.** Effect of Length of Plants *Phaseolus vulgaris* L. inoculated with bacterial biofertilizers

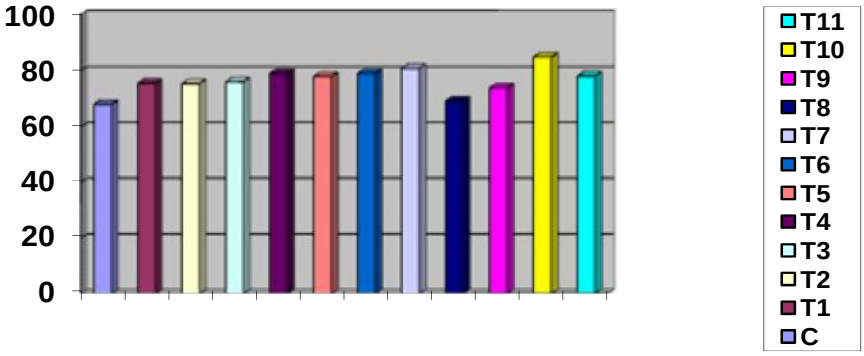



**Figure 5** . Effect of Shoot length *Phaseolus vulgaris* L. inoculated with bacterial biofertilizers

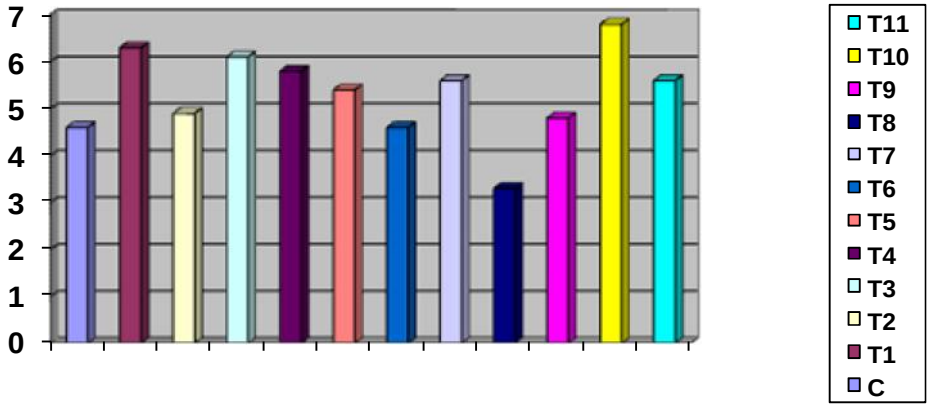


**Figure 6.** Effect of Root length *Phaseolus vulgaris* L. inoculated with bacterial biofertilizers

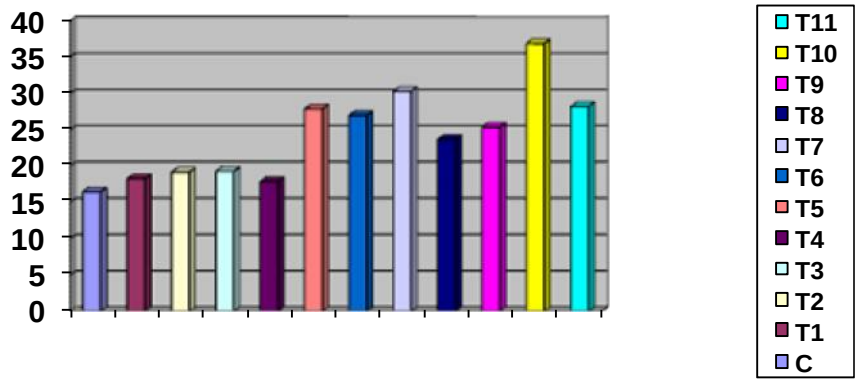



**Figure 7 .** Effect of Total length of plants *Phaseolus vulgaris* L. inoculated with bacterial biofertilizers

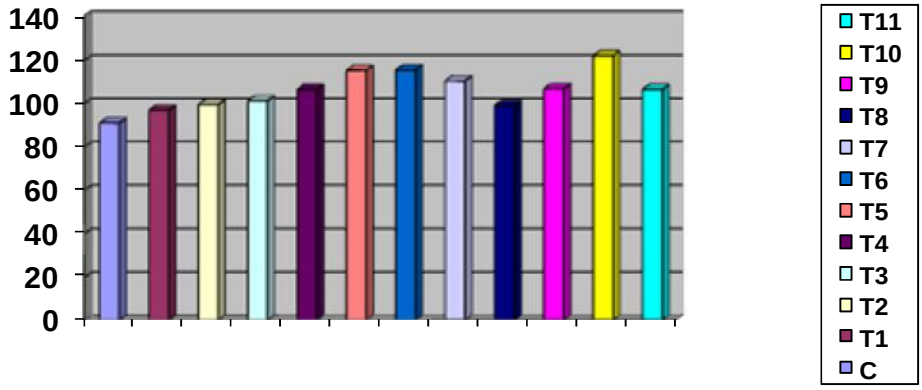



**Figure 8.** Effect of Reducing sugars *Phaseolus vulgaris* L. inoculated with bacterial biofertilizers

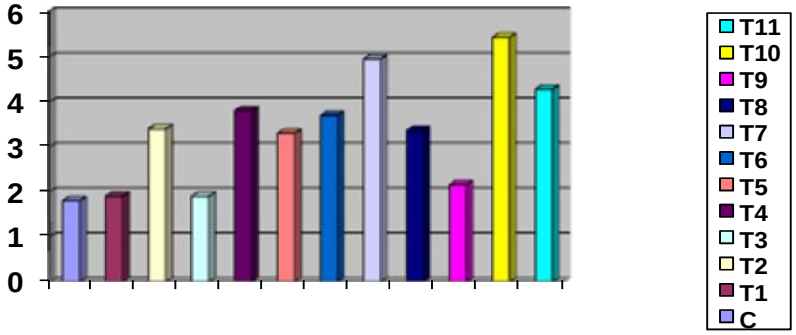
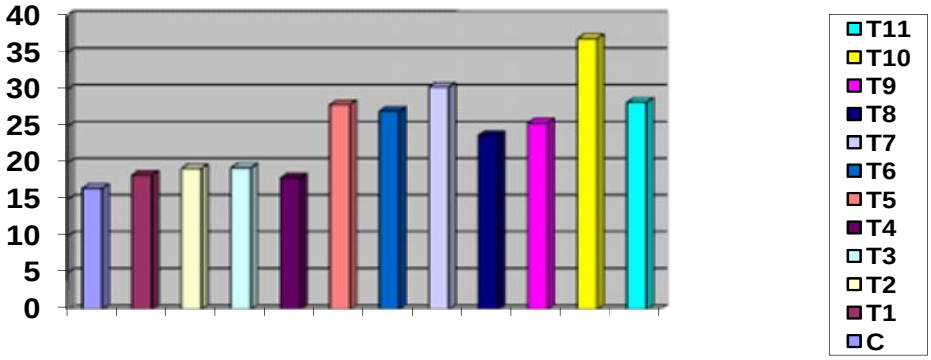


**Figure 9.** Effect of No. of flowers *Phaseolus vulgaris* L. inoculated with bacterial biofertilizers




**Effect on total free amino acids**

The total free acids of *Phaseolus vulgaris 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 *Phaseolus vulgaris L.* plants were 18.46 mg/g. 15.75 (*Rhizobium* sp, *Azospirillum* sp and Phosphobacteria), 5.10 (*Rhizobium* sp.) and 2.25 respectively on 50 DAS (Table. 4; Figure. 13).

**Effect on reducing sugar**

The reducing sugar content on *Phaseolus vulgaris L.* with combined treatments of *Rhizobium* sp., Phosphobacteria,*Azotobacter* sp and *Azospirillum* sp. was found to be 5.43 mg/100g, 4.95 in dual (Phosphobacteria, *Rhizobium* sp 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, *Azotobacter* sp and *Azospirillum* sp. combined treatments of *Phaseolus vulgaris L.* plants, the inorganic phosphorus contents were showed maximum than dual, alone and control plants.The increase in inorganic content was observed in *Phaseolus vulgaris L.* plants of combined treatments were 6.17 mg/g, 5.90 (*Rhizobium* sp and *Azotobacter* sp, Phosphobacteria)on 50 DAS (Table. 4 ;Figure. 15).

**Figure 10.** Effect of Chlorophyll content of plants *Phaseolus vulgaris* L. inoculated with bacterial biofertilizers

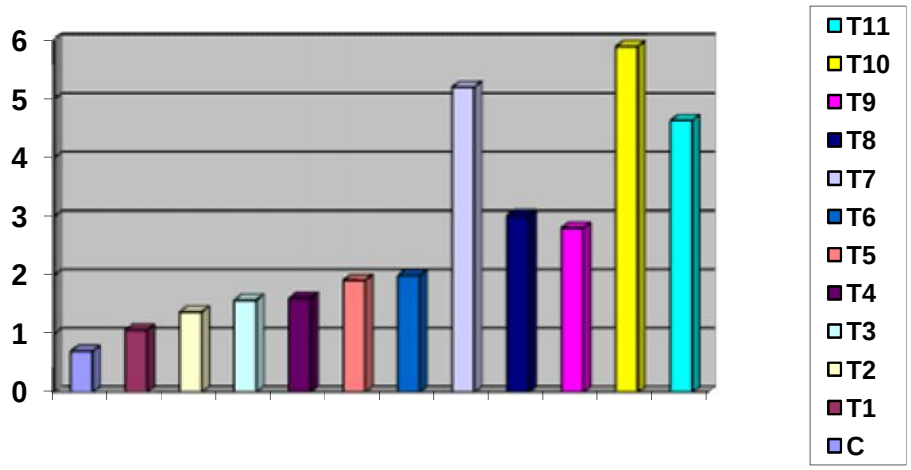


**Figure 11.** Effect of Protein *Phaseolus vulgaris* L. inoculated with bacterial biofertilizers

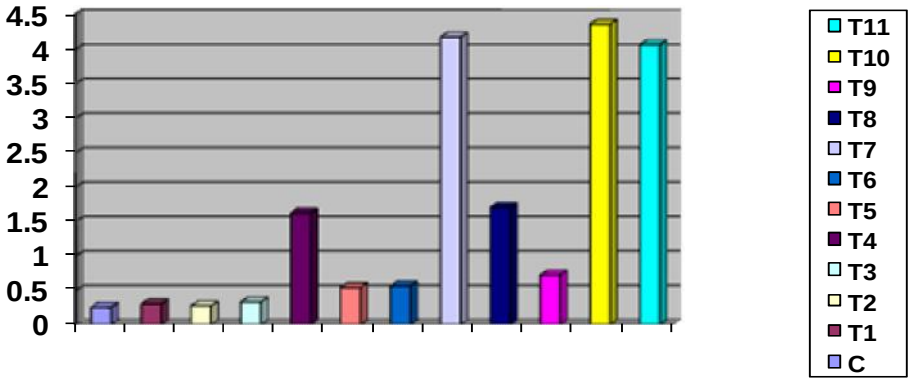



**Figure 12.** Effect of Carbohydarate content of plants *Phaseolus vulgaris* L. inoculated with bacterial biofertilizers

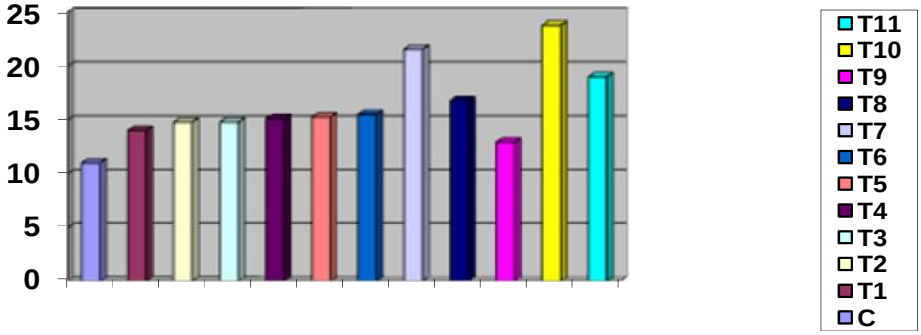



**Figure 13.** Effect of total free amino acids *Phaseolus vulgaris* L. inoculated with bacterial biofertilizers

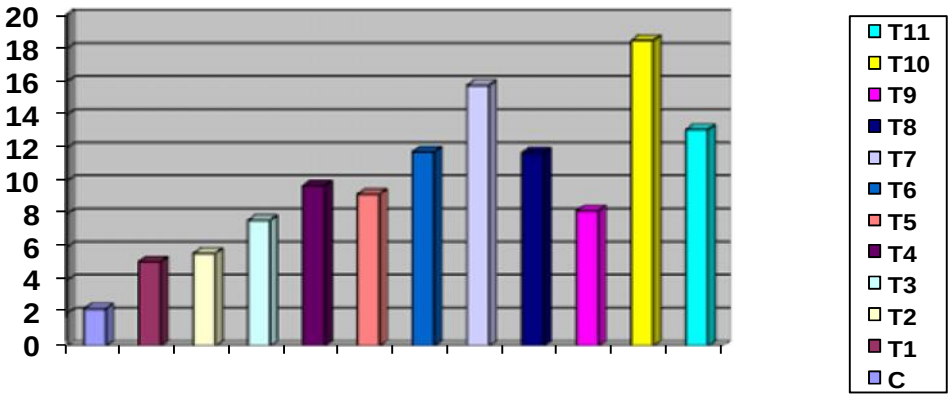


**Figure 14.** Effect of inorganic phosphorus content of plants *Phaseolus vulgaris* L. inoculated with bacterial biofertilizers

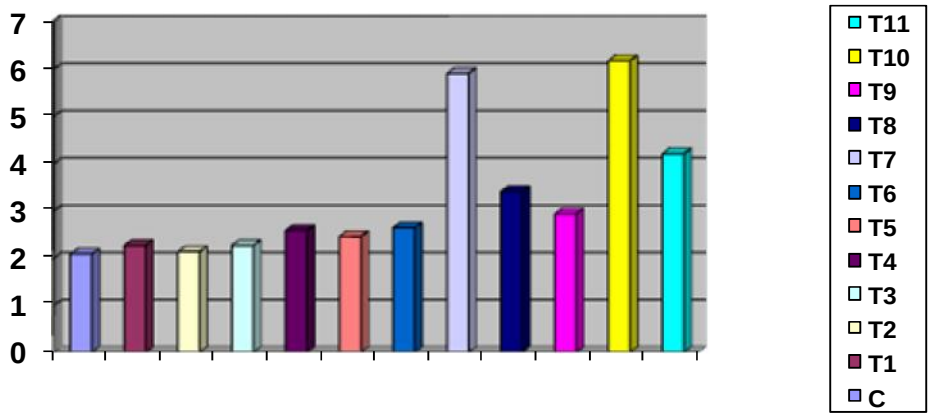
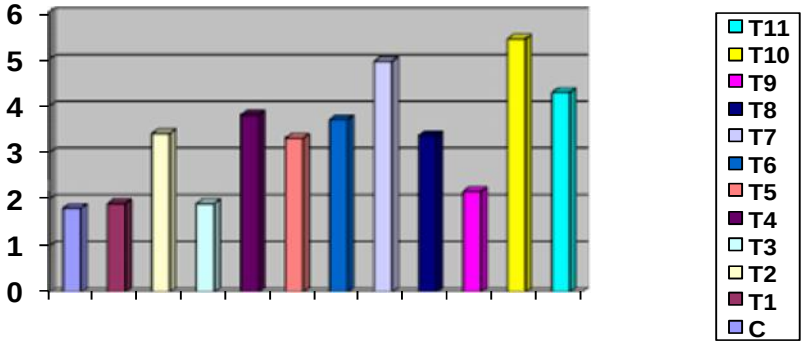



**Figure 15.** Effect of Reducing sugars *Phaseolus vulgaris* L. inoculated with bacterial biofertilizers

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