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Research Article

Effect of bacterial biofertilizer on growth of *Lablab purpureus* L. Plants

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Abstract

The present investigation was carried out to study the effect of bacterial Biofertilizers on pulse crop like *Lablab purpureus* L. plants. The microbial inoculants were sowed in sterile polythene bags containing sterilized soil samples. Controls were also maintained without a bacterial biofertilizers. After 40 days of sowing, the plant growth parameters like morphological and Bio-chemical parameters were analyzed in *Lablab purpureus L.* 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., Phosphobacteria and *Azospirillum* sp. *Lablab purpureus 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 and *Azospirillum* sp. plants of *Lablab purpureus L.*. than dual inoculation and control plants.

Keywords: bacterial Biofertilizers ; *Lablab purpureus* L.; morphological and Bio-chemical parameters.

Introduction

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.

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. 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.

Tien *et al.*, (1979) have attributed that the increased yield in pear millet obtained by inoculation of *Azospirillum* was due to the production of indole acetic acid, gibberellins and cylokinins like substances by the bacterium and their subsequent effect on the plant. Biofertilizer play a main key role for selective absorption of immobile (P, Zn, and Cu, and mobile (C, S, Ca, K, Mn, CL, Br and N) elements to plants. These are all available to plant in less amount was separated by Tinker (1984).

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. Subba Rao and Singh (1985) reported that the increased the grain yield over the control with the treatments of *Azospirillum brasilense*, urea (alone) and Urea +*A.brasilense*. Tilak and Subba Rao (1987) studied the effect of seed inoculation with *A.brasilense* in near millet plants.

Pulse crops have been an important component of agriculture since ancient times. These leguminous plants of symbiotic association with soil bacterium, the *Rhizobium* forms nitrogen fixing root nodules which are agronomically significant as they provide an alternative to the use of energy expensive nitrogenous chemical fertilizer. Despite the large and increasing use of nitrogenous fertilizers in agriculture, estimates suggest that biological nitrogen fixation contributes at least four times more nitrogen to the soil throughout the world. On a global basis these symbiotic association between legume and *Rhizobium* may reduce about 70 million tons of atmospheric nitrogen to ammonia per annum which amounts to about 40% of all biologically fixed nitrogen per year (Burns and Hardy, 1975.)

Aim and objectives

Effect of Bacterial biofertilizers (*Rhizobium* sp., Phosphobacteria and *Azospirillum* sp.) on different growth parameters of *Lablab purpureus* 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 *Lablab purpureus* L. Biofertilizers such as *Rhizobium* sp., Phosphobacteria and *Azospirillum* sp. were isolated from soil samples and used as inoculums.

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.

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).

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 *Lablab purpureus 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 was taken 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 *Lablab purpureus L.*. plants treated with Rhizobium sp. T2-seeds of *Lablab purpureus L.*. plants**.** treated with *Azospirillum* sp. T3-seeds of *Lablab purpureus L.*. plants**.** treated with *Phosphobacterium* sp. T4 - seeds of *Lablab purpureus L.*. plants**.** treated with Rhizoium and *Azospirillum* sp. T5-Seeds of *Lablab purpureus L.*. plants**.** treated with *Rhizobium* sp. and *Phosphobacteria* sp. T6 -seeds of *Lablab purpureus L.*. plants**.** treated with *Azospirillum* sp. and Phosphobacteria T7-seeds of *Lablab purpureus L.*. plants**.** treated with *Rhizobium* sp., Phosphobacteria and *Azospirillum* sp. T8- seeds of *Lablab purpureus L.*. plants**.** treated Urea and *Azospirillum* sp. T9- seeds of *Lablab purpureus L.*. plants**.** treated Neem Cake. T10- seeds of *Lablab purpureus L.*. plants**.** treated FYM, *Rhizobium* sp., Phosphobacteria and *Azospirillum* sp. T11- seeds of *Lablab purpureus L.*. plants**.** treated Neem Cake, *Rhizobium* sp., Phosphobacteria and *Azospirillum* sp. After 40 days of sowing the morphological and bio-chemical parameters of *Lablab purpureus* 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 *Lablab purpureus 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 *Lablab purpureus* L. 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 (Plates. 3,4&5).

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

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

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	15.8	3.6	4.6	68.1	55.0
Rhizobium sp.	17.6	5.4	6.3	75.6	60.1
Azospirillum	15.9	5.8	4.9	70.4	63.4
Phosphobacteria sp.	16.8	6.4	6.1	76.1	59.4
Rhizobium sp. +*Azospirillum*	17.8	5.9	5.8	79.1	69.1
Rhizobium sp. + Phosphobacteria sp.	19.4	7.6	5.4	78.0	72.1
Azospirillum sp .+Phophobacteria	18.3	5.9	4.6	79.1	72.1
Rhizobium +Phophobacteria +*Azospirillum*	22.0	8.1	5.6	80.9	76.9
Urea + Azospirillum sp.	17.3	6.2	3.3	69.2	56.5
Neem Cake	16.4	8.8	4.8	73.9	70.9
FYM+ Rhizobium +Phophobacteria +*Azospirillum*	24.0	9.2	6.8	85.0	79.2
Neem Cake +Rhizobium +Phophobacteria +*Azospirillum*	19.0	8.0	5.6	78.2	73.0

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

Treatments	Number of nodules/plant	Number of	Root length (cm)	Total length of plant (cm)
Control	8.4	6	16.8	90.9
Rhizobium sp.	12.2	8	18.3	96.8
Azospirillum	12.0	7	19.2	99.4
Phosphobacteria sp.	9.0	6	19.3	101.1
Rhizobium sp. +*Azospirillum*	12.0	8	17.9	106.4
Rhizobium sp. + Phosphobacteria sp.	18.2	7	27.9	115.1
Azospirillum sp .+Phophobacteria	21.0	6	27.0	115.3
Rhizobium +Phophobacteria +*Azospirillum*	25.0	10	30.3	110.3
Urea + Azospirillum sp.	13.0	4	23.7	98.9
Neem Cake	11.0	5	25.4	106.7
FYM+ Rhizobium +Phophobacteria +*Azospirillum*	33.0	11	36.9	121.9
Neem Cake +Rhizobium +Phophobacteria +*Azospirillum*	18.0	7	28.2	106.4

Table.3 Effect of biochemical parameters of *Lablab purpureus* 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	1.37	0.27	14.80
Phosphobacteria sp.	1.57	0.33	14.80
Rhizobium sp. +*Azospirillum*	1.62	0.62	15.11
Rhizobium sp. + Phosphobacteria sp.	1.91	0.54	15.27
Azospirillum sp .+Phophobacteria	1.99	0.56	15.51
Rhizobium +Phophobacteria +*Azospirillum*	5.21	4.17	21.57
Urea + Azospirillum sp.	3.01	1.07	16.78
Neem Cake	2.80	0.72	12.94
FYM+ Rhizobium +Phophobacteria +*Azospirillum*	5.89	12.36	23.80
Neem Cake +Rhizobium +Phophobacteria +*Azospirillum*	4.63	4.06	19.02

Table.4 Effect of biochemical parameters of *Lablab purpureus* L. plants 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	3.40	5.60	2.13
Phosphobacteria sp.	1.90	7.60	2.26
Rhizobium sp. +Azospirillum	3.80	9.69	2.58
Rhizobium sp. + Phosphobacteria sp.	3.30	9.18	2.45
Azospirillum sp .+Phophobacteria	3.70	11.73	2.64
Rhizobium +Phophobacteria +Azospirillum	4.95	15.75	5.90
Urea + Azospirillum sp.	3.36	11.68	3.40
Neem Cake	2.16	8.17	2.93
FYM+ Rhizobium +Phophobacteria +Azospirillum	5.43	18.46	6.17
Neem Cake +Rhizobium +Phophobacteria +Azospirillum	4.28	13.08	4.20

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

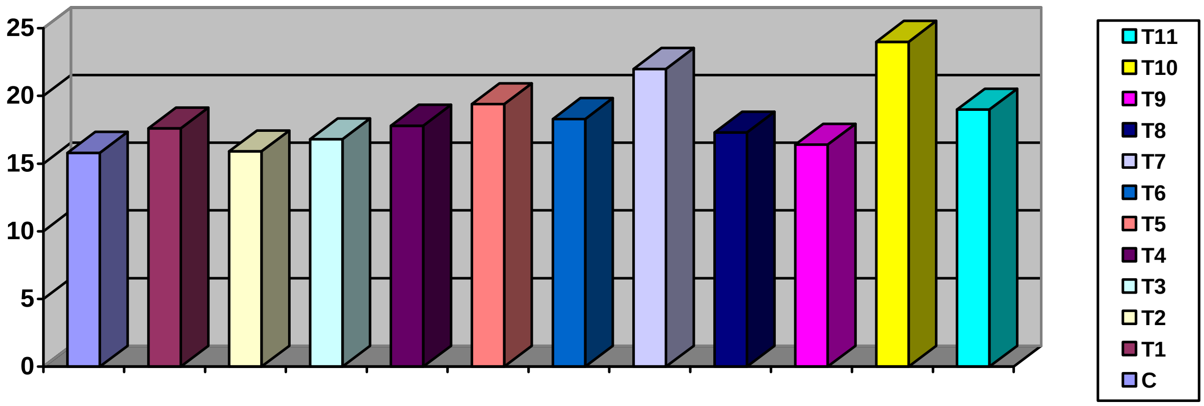


Figure.2 Effect of Number of leaves *Lablab purpureus* L. inoculated with bacterial biofertilizers

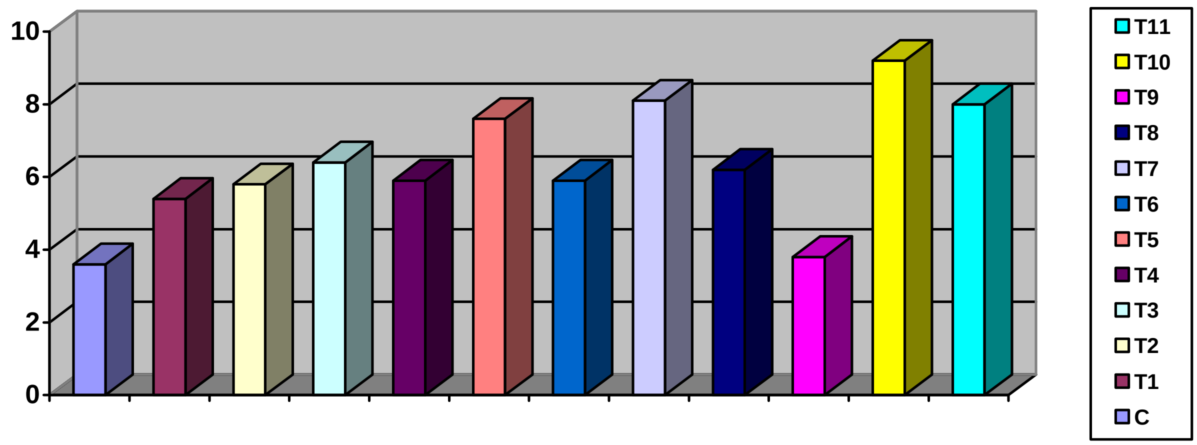


Figure.3 Effect of Breadth of leaves *Lablab purpureus* L. inoculated with bacterial biofertilizers

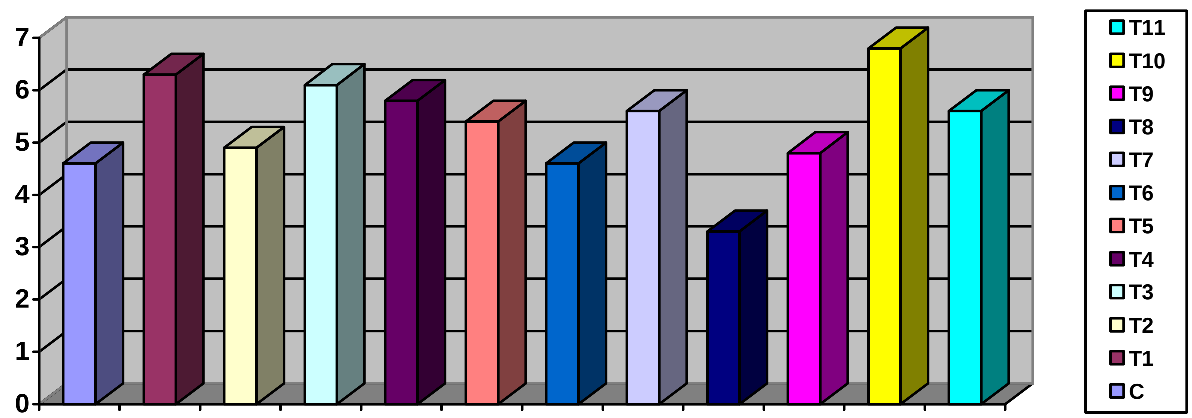


Figure.4 Effect of Length of Plants *Lablab purpureus* L. inoculated with bacterial biofertilizers

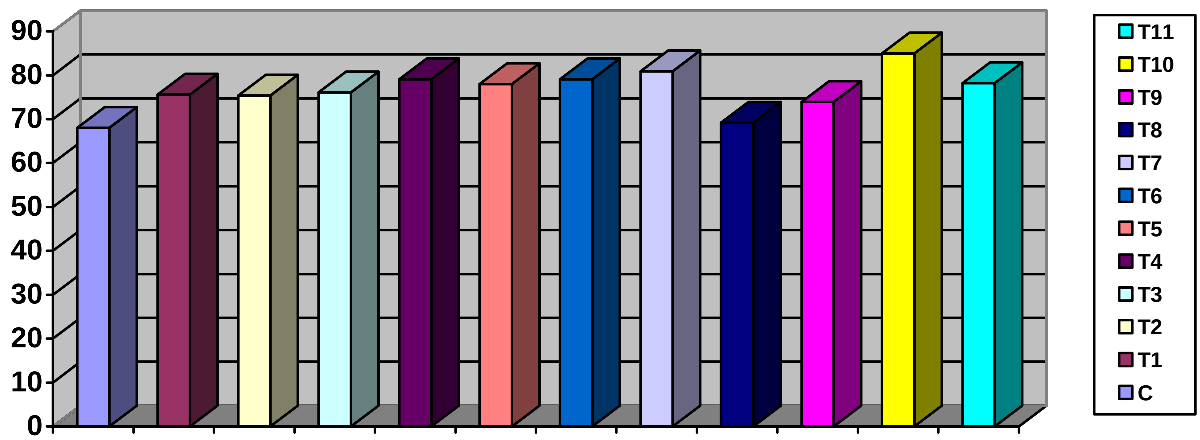


Figure.5 Effect of Shoot length *Lablab purpureus* L. inoculated with bacterial biofertilizers

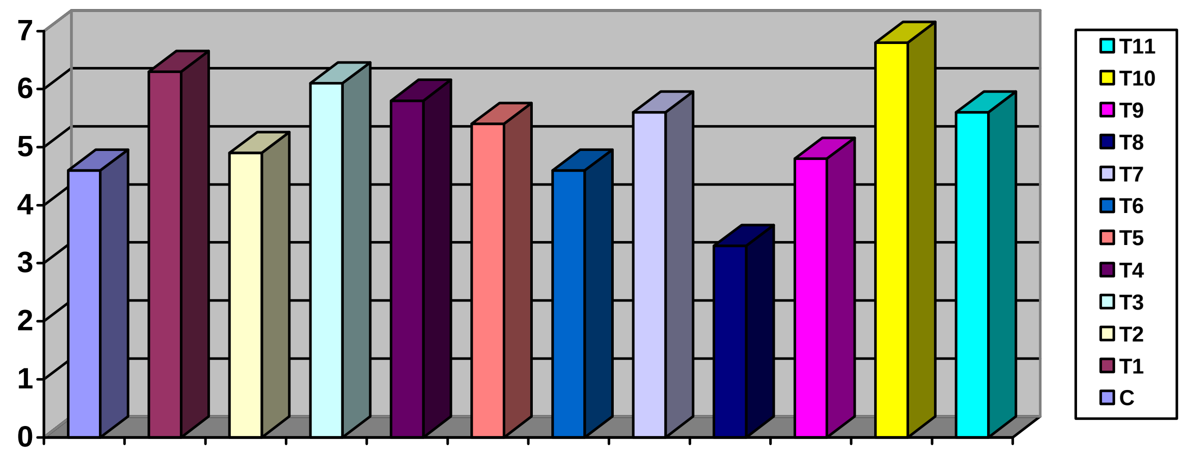


Figure.6 Effect of Root length *Lablab purpureus* L. inoculated with bacterial biofertilizers

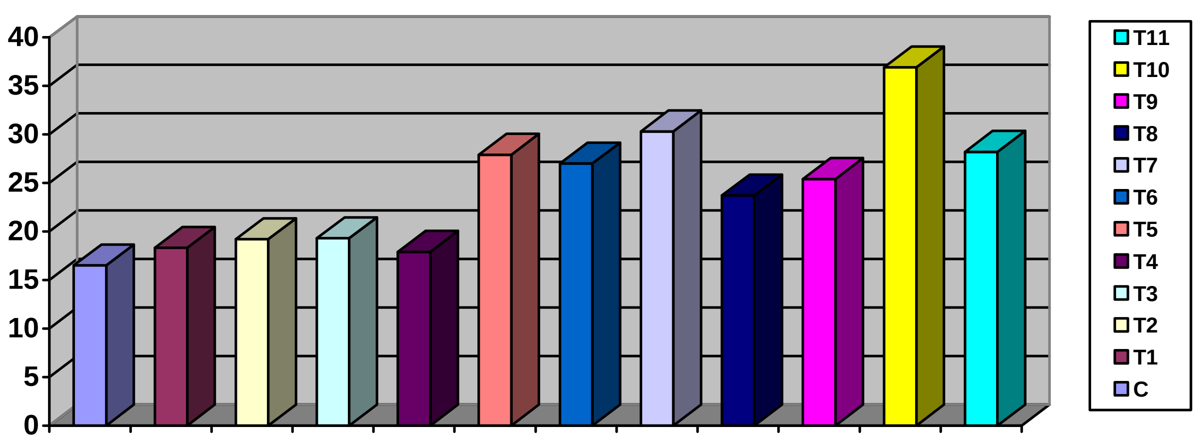


Figure.7 Effect of Total length of plants *Lablab purpureus* L. inoculated with bacterial biofertilizers

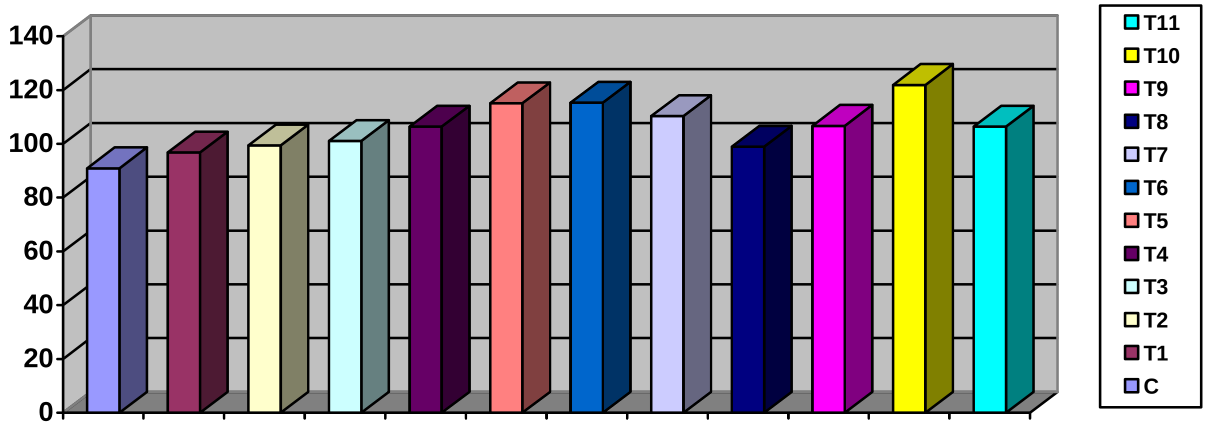


Figure.8 **Effect of Reducing sugars *Lablab purpureus* L. inoculated with bacterial biofertilizers**

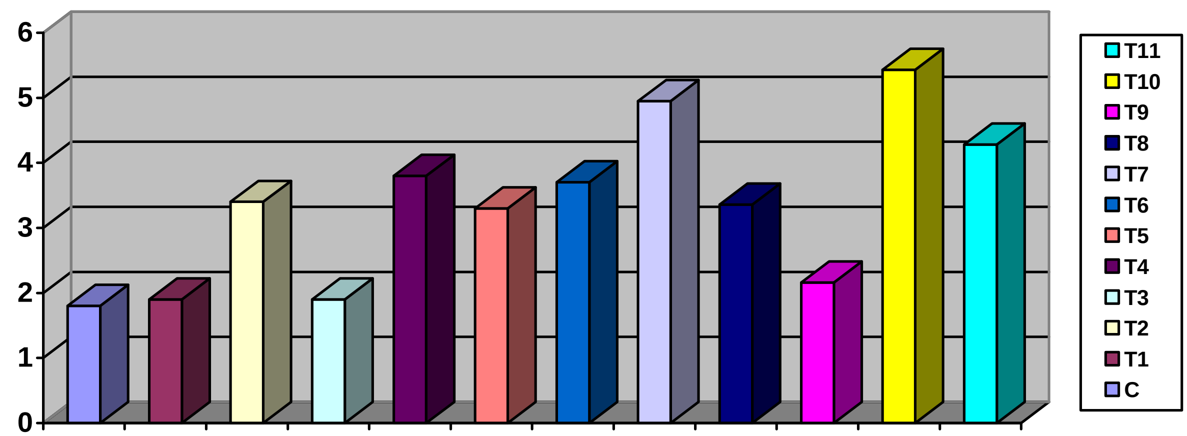


Figure.9 Effect of No. of flowers *Lablab purpureus* L. inoculated with bacterial biofetilizers

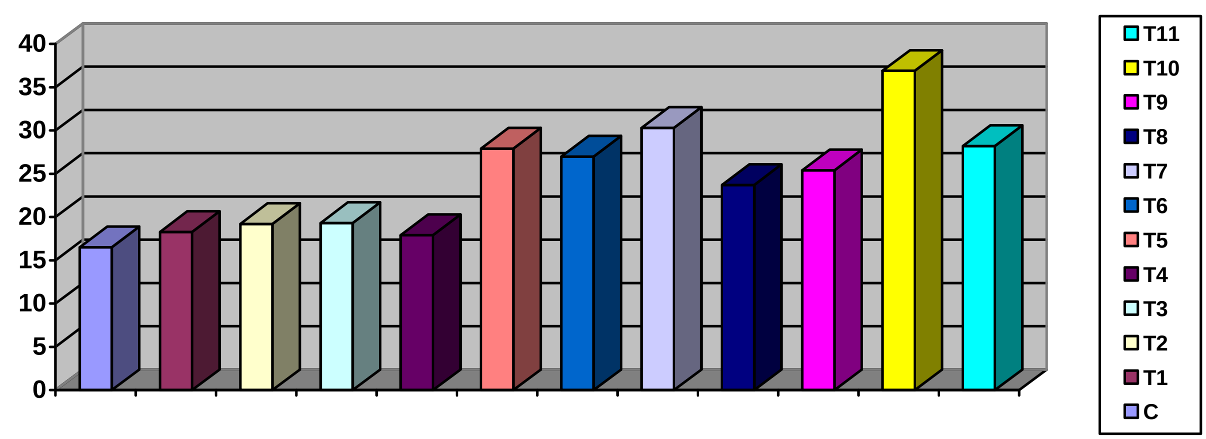


Figure.10 Effect of Chlorophyll content of plants *Lablab purpureus* L. inoculated with bacterial biofertilizers

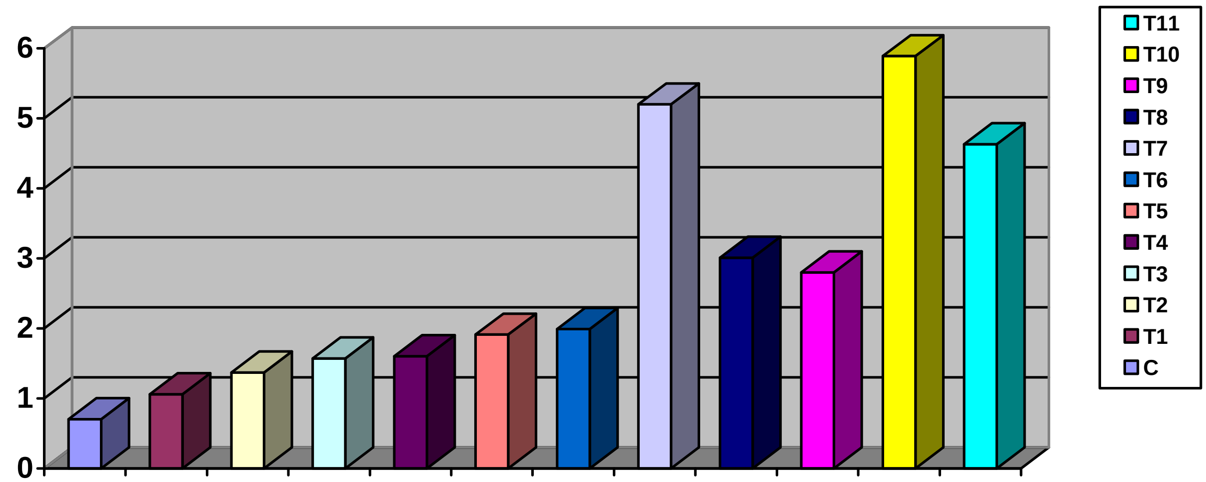


Figure .11 Effect of Protein *Lablab purpureus* L. inoculated with bacterial biofertilizers

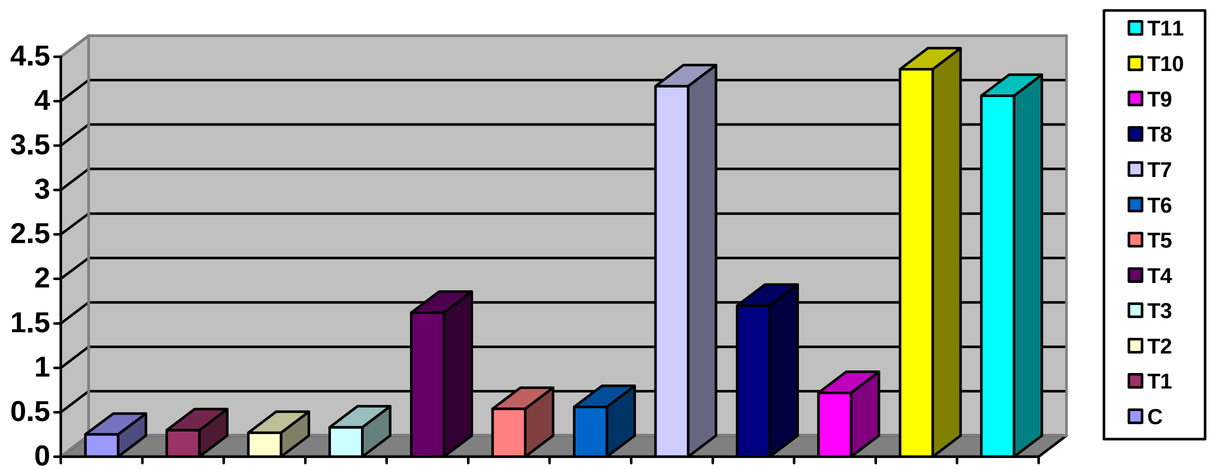


Figure.12 Effect of Carbohydarate content of plants *Lablab purpureus* L. inoculated with bacterial biofertilizers

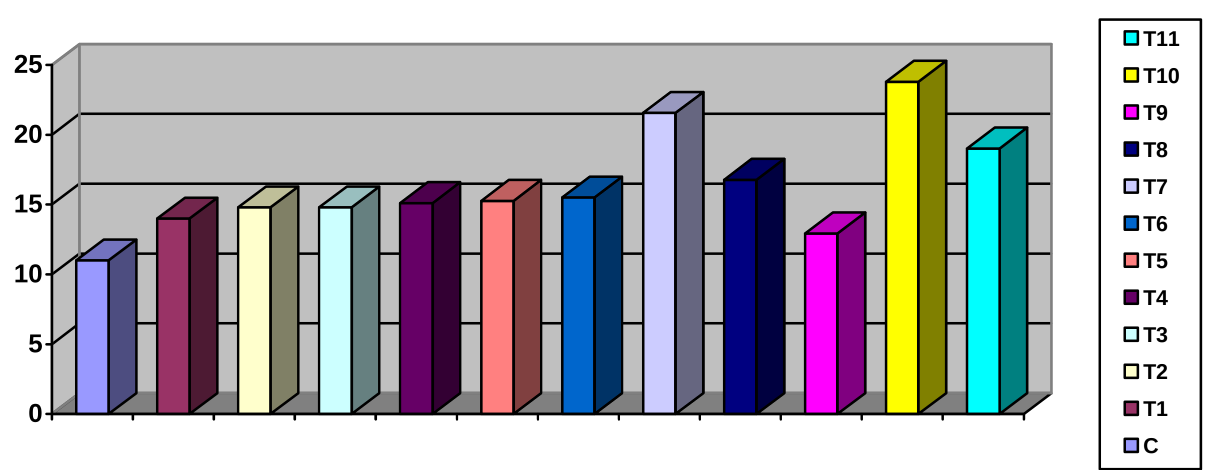


Figure.13 Effect of total free amino acids *Lablab purpureus* L. inoculated with bacterial biofertilizers

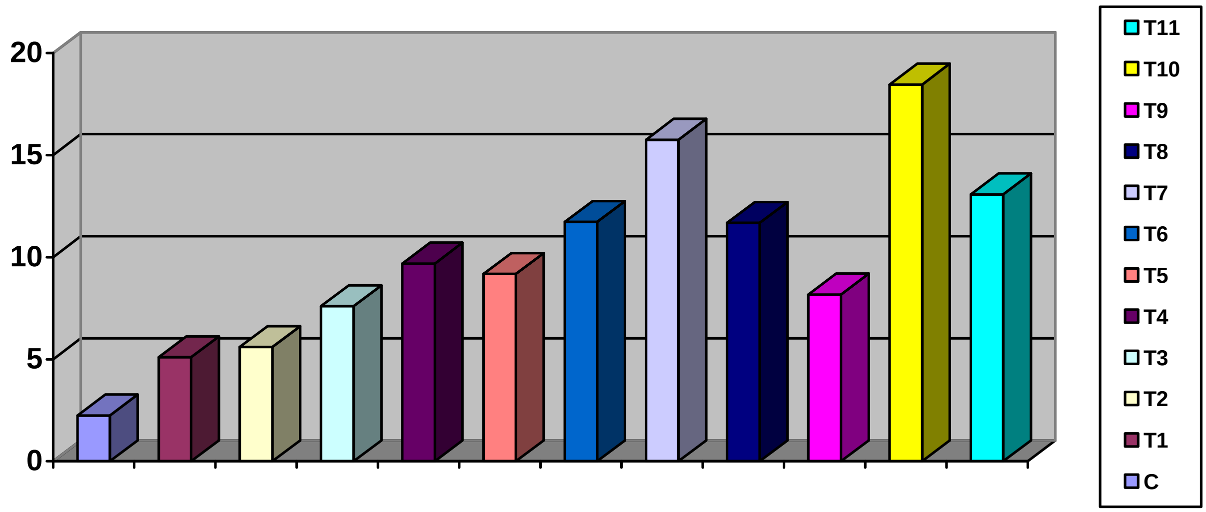


Figure.14 Effect of inorganic phosphorus content of plants *Lablab purpureus* L. inoculated with bacterial biofertilizers

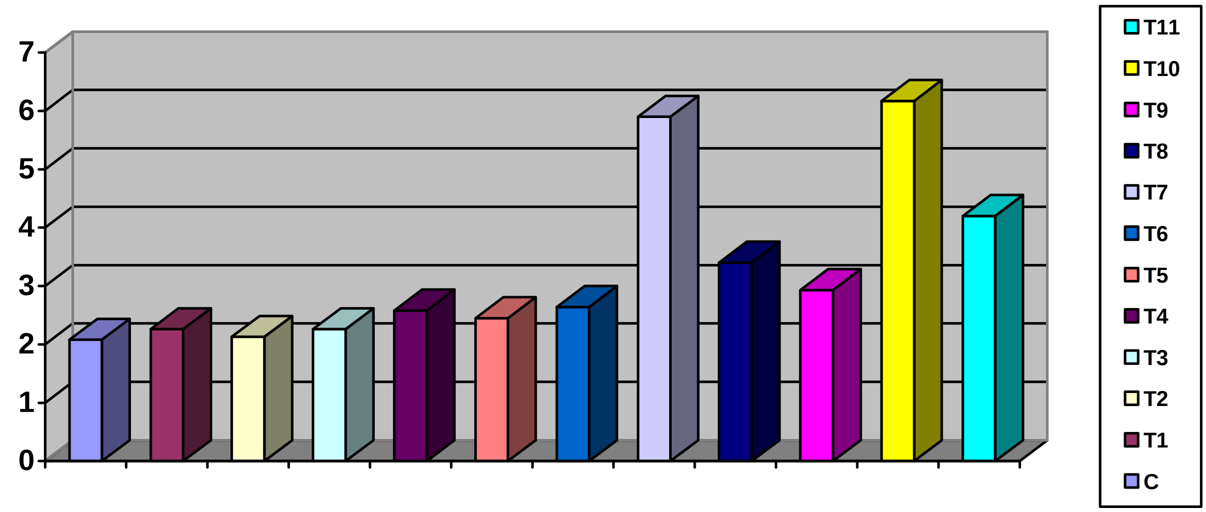
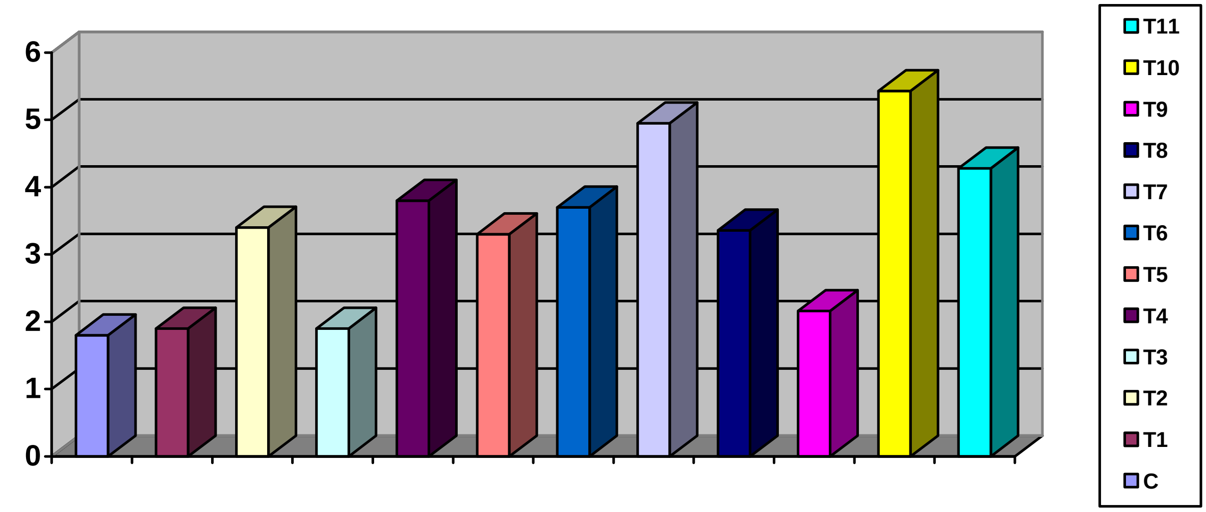


Figure.15 Effect of Reducing sugars *Lablab purpureus* L. inoculated with bacterial biofertilizers



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).

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