

## INFLUENCE OF BIO FERTILIZERS ON PLANT GROWTH OF BEAN (*Lablab purpureus* (L).

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### ABSTRACT

The present study was elucidated the effects of bio fertilizers on plant growth of (*Lablab purpureus* (L). COGB14 Verity. The research was conducted during the Rabi season 2016 to 2017. At the field experimental centre Department of Botany, Arignar Anna Arts and Science College, Villupuram, Tamil Nadu. The data were recorded on morphological characters based on the mean performance of the treatment (T4 -AMF+AZOS+AZOTO) was found best treatment for plant growth. Compare to the other treatments and control. The obtained results showed that the germination percentage, shoot length, root length, fresh weight, dry weight and leaf area. Interaction effect of bio fertilizers was significant for all characters. Thus it, indicates that the process of bio fertilizers, may be better option for the seed growers, to achieve seed yield and yield components in (*Lablab purpureus* (L). COGB14 Verity.

**Keywords:** Arbuscular mycorizha, Endophytic, bio fertilizers, Interaction

### 1. INTRODUCTION

Beneficial interactions of plant and microbe that promote plant health and development have been the subject of considerable study. At the most basic level, endophyte simply means the location of an organism, with “endo” means “inside” and “phyte” means “plants”. Therefore, endophyte refers to organisms that live within plants. Bacteria and fungi are the most common organisms associated with the term endophyte. Endophytic organisms associated with plants are varied and complex. Endophytic microbes occupy a relatively privileged niche within plant and usually contribute to plant health. Some groups of endophytic microorganisms have been believed to be mutualists that protect plants against biotic stresses. Co-evolution may exist between endophytes and their host in resist to environmental stresses. Endophytic bacteria help to enhance the nutrient availability and fix nitrogen for plants. Recently, studies have indicated that bacteria colonizing the plant interior are able to improve plant growth and suppress pathogen. Competitions between endophytic bacteria and pathogens are observed due to the limited nutrient supply inside plant tissue. Although the facts of endophytic bacteria reducing the negative impact of plant pathogen have been well documented, few reports indicate that plant pathogens have significant influence on endophytic bacteria. Early evidence for non-symbiotic nitrogen fixation

was provided by studies of N balance in various ecological studies. Different crops and grasses, such as sugar cane in Brazil, wetland rice in Asia, and some cereal field in Canada, were observed growing healthy without artificial nitrogen input. Those plants were thought to benefit from the endophytic nitrogen fixers. Thus, the nitrogen fixation bacteria associated with non-legume plant caught people's attention. The ecological and economic importance of nitrogen fixation in rhizobium-legume symbiosis has earned research attention for long time. In legumes, the endophytic bacteria - rhizobium stimulates the plants to develop root nodules. In root nodules, the bacteria inhabit and construct a co-metabolic system with the legumes; so as to form a well-developed symbiosis. This process is highly efficient and provides significant proportion of plant nitrogen. Recent work has proved that significant N fixation processes also exist in some legumes, particularly sugar cane, rice and maize. The study on endophytic diazotrophic bacterium-Azospirillum is a growing interest in diazotrophic bacteria associated with graminaceous plants was manifested all over the world. Azospirillum spp. These spread from plant generation to plant generation via seeds, vegetative propagation and dead plant material and possibly by sap feeding insects. On the other hand, Azospirillum enters host plants via seeds or wounds. Bacterial mechanisms of plant growth promotion include biological nitrogen fixation (BNF), synthesis of phytohormones, environmental stress relief, synergism with other bacteria-plant interactions, inhibition of plant ethylene synthesis, as well as increasing availability of nutrients like

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phosphorus, iron and minor elements, and growth enhancement by volatile compounds. Soil is the habitat for plant roots, micro flora (bacteria, actinomycetes, fungi and algae), micro fauna and macro fauna. The zone of soil under the influence of root is called the "rhizosphere". This area of activated microbial populations can extend more than 5 mm from the root surface. It is now recognized that the "rhizosphere effect" is mainly due to the exudates from the roots, which attract soil microorganisms. These microorganisms play vital roles in physiological processes in the ecosystem of plants growing in soil. The rhizosphere bacteria, actinomycetes and fungi carry out a range activity of great relevance to the plant growth, plants disease development or protection of plants from the pathogens. Based on these relationship of microorganisms with plant's root, the rhizosphere organisms can be categorized into saprotrophs, usually opportunists leaf benefactors in some situation parasitic symbionts or pathogens, potentially harmful to the plants; and mutualistic symbionts which develop activities beneficial to plant growth. These microorganisms interact with both plant roots and soil constituents in the rhizosphere.

Many PGPR (Plant Growth Promoting Rhizobacteria) show great promise as potential inoculants for agricultural uses and environmental protection and play critical role in maintaining the sustainability of agro ecosystems. However, the current use of PGPR in agriculture is somewhat poor despite numerous reports on their proven performance under laboratory conditions. PGPR possess the ability to colonize and establish an on-going relationship with plants, resulting in better root growth, more biomass, and a substantial increase in crop yield. In this context, significant effects of PGPR have been observed on various agricultural crops, including legumes, cereals, and noncereals, and some other important plant species. The most important interactions developing among plant and microbes in the rhizosphere can be classified into three main groups, plant-plant interactions caused by overlapping rhizospheres, which results in competition for nutrients, root-microorganisms interactions, determined by plant activities that stimulate microorganisms to grow around the roots (rhizosphere effect) and by microbial activities that affect plant development, either by benefiting the plants or by inducing disease, and microbe-microbe interactions, which include both synergistic and antagonistic activities

#### **Lablab bean (*Lablab purpureus* L).**

Lablab bean is a twining vine with leaves divided into 3 leaflets, and attractive bright purple flowers and purple pods. The vine becomes woody and can reach more than 30 Ft. in length. The leaflets are purplish green, broad oval or triangular in shape and 3-6 in long. Flowers are typically shaped like pea flowers, a rich, brilliant purple and arranged in loose clusters on long stems that extend above the foliage. For this reason, Lablab bean is frequently grown as an ornamental vine. The pods are just as showy as the flowers. They are flat and curved, about 3 in long, bright purple. The beans inside are dark It is a perennial herb, frequently grown as an annual. Usually twining to reach 5-29 feet, but bushy, semi-erect, and prostrate forms exists. Probably no other legume shows such variation in form and habit. The tap-root is well developed with many laterals and well developed

adventitious roots. Purse glove 1968 has recognized two types of Lablab in India and is sometimes considered as distinct species. They are: *Dolichos lablab* var. *typicus* Prain: It is commonly called Lablab bean, Bonavist bean, Hyacinth bean, Indian butter bean (Hindi-Sem; Bengali-Shim; Gujarathi-Val; Marathi-Pavta; Telugu-Chikkudu; Tamil-Avarai; Kannada-Chapparadavare; Malayalam- Avara.

A perennial twining herb, cultivated mostly as an annual, distributed throughout the tropical and temperate regions of Asia, Africa and America. In India, it is grown as a garden crop. Several types differ in colour of flowers, size, shape and texture of pods and size and colour of seeds. A type with showy purple flowers is cultivated as an ornamental plant in temperate regions. The pods are white, green or purple-margined. Seeds white, yellow, brownish, purple or black. *Dolichos lablab* var. *lignosus* Prain: It is commonly known as Australian pea, Field Bean. (Hindi - Ballar; Gujarati - Val; Telugu - Anumulu; Tamil - Mochai; Kannada - Avare; Malayalam - Mochakotta). It is a semi-erect, bushy, perennial herb, cultivated as an annual. It shows little or no tendency to climb. Leaflets innately trifoliate, smaller than those of var. *typicus*. Flowers borne on a straight upright stalk, often a foot high on which they open in succession. Pods oblong, flat and broad, firm-walled and fibrous contain 4-6 seeds with their long axis at right angles to the suture.

Seeds almost rounded white, brown or black. The plant emits a characteristic odour. lored with a conspicuous white eye. The pod is widely eaten in India. It is very difficult to estimate the total size of green inputs market in India because of its diversity in terms of products and also due to the nature of it being unorganized market. Green inputs into agriculture include bio-fertilizers, bio-pesticides, compost, Farm Yard Manure (FYM), green manure etc. As most of this inputs are either not traded and even if they are traded, it is only at informal levels available information regarding production capacity, demand and sales is at best sketchy estimation and hence inadequate. The limited number well-established firms that have their presence in this market today as a result it this is predominately controlled by the small and local producers of bio-fertilizer, vermicompost and other input producers who are in large numbers. Biofertilizers are low cost, renewable sources of plant nutrients. These are selected strains of beneficial soil microorganisms cultured in the laboratory and packed in suitable carrier. Increased crop production largely relies on the type of fertilizers used to supplement essential nutrients for plants. This investigation aimed at studying the interaction effect of Arbuscular Mycorrhizal Fungi (AMF), azospirillum and azotobacter that are likely to possess agronomical beneficial traits viz, N-fixation, P-mobilization and production of plant growth substances for the lablab bean (*Lablab purpureus* L.) crop.

## **2.MATERIALS AND METHODS**

The field experiment was conducted at the Department of Botany, Arignar Anna Arts and Science College, Villupuram, Tamil Nadu. The land was harrowed well and brought to the good tilth. The land was divided into blocks at 1m apart and micro plots of 4.2×2.8m<sup>2</sup> were prepared with in each block. 50 cm distance was maintained between two microplots with in each the treatments were allotted randomly. Each micro

plot was surrounded by bund in order to prevent the mixing up inoculum. Lablab bean *Lablab purpureus* (L). Seeds were sowing micro plots with 60cm× 75cm spacing, respectively. The weather is moderately warm with hot summer months.

## MATERIALS

### Cultivar

The seeds of experimental crop Lablab bean COGB14 *Lablab purpureus* (L). Were obtained from Department of vegetable crops Tamil Nadu Agricultural University (TNAU), Coimbatore, Tamil Nadu, and India.

### Bio fertilizers

Bio fertilizers AM Fungi, Azospirillum, and Azotobacter was obtained from the Department of Microbiology Tamil Nadu Agricultural University (TNAU), Coimbatore, Tamil Nadu, India.

## METHODS

### EXPERIMENTAL DESIGN AND TREATMENTS DETAILS

The experiment design and details are as follows,

Cultivar - Lablab bean COGB14 *Lablab purpureus* (L).

No. of treatments -5

Sampling days – 7<sup>th</sup>, 15th, 30th, 45th, 60th, 90th DAS

T1: AM Fungi alone  
T2: Azospirillum alone  
T3: Azotobacter alone  
T4: AMF+AZOS+AZOTO+  
T5: Control (uninoculated)

### GERMINATION STUDIES

Lablab bean COGB14 *Lablab purpureus* (L) seeds were surface sterilized with 0.2 per cent of HgCl<sub>2</sub> for two minutes and they were thoroughly washed with tap water. The seeds were equispacially arranged in plastic cup filled with garden soil and they were treated with different Bio fertilizers such as AM Fungi, Azospirillum, Azotobacter and AMF+AZOS+AZOTO. The e control set was maintained by using tap water. Five replicates were maintained for each treatment seventh day, the germination percentage, shoot length, root length, total leaf area, seedling fresh weight, seedling dry weight were taken. From these data, the following values of vigor index, and tolerance index were calculated.

### Germination percentage

The number of seeds germinated in each concentration was counted on the 7<sup>th</sup> day and the germination percentage was calculated by using the following formula

$$\text{Germination percentage} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds sown}} \times 100$$

### Shoot and root length (cm/seedling)

Twenty seedlings were taken from each treatment and their shoot length and root length were measured by using a cm scale and the values were recorded.

### Total leaf area

The total leaf area was calculated by measuring the length and width of the leaf as described by Yoshida *et al.* (1972).

$$\text{Leaf area (cm}^2\text{)} = K \times \text{length} \times \text{breadth}$$

Where

$$K = \text{Kemp's constant (for dicot leaves 0.66)}$$

### Fresh weight (g/seedling)

Ten seedlings were collected from each treatment and their fresh weights were measured with the help of an electrical single pan balance.

### Dry weight (g/seedling)

The same seedlings used for fresh weight were kept in hot air oven at 80°C for 24 hours. Then, the seedlings were taken from the oven and kept in desiccators for some time. Their dry weights were taken by using an electrical single pan balance.

## 3.RESULTS

The present investigation deals with the effects of various Bio fertilizer treatments on seed germination, growth parameters such as shoot length, root length, fresh weight, dry weight and total leaf area of components of Lablab bean (*Lablab purpureus* L). The results are presented in this chapter. The influence of AM fungi Azospirillum and Azotobacter on the plant lablab bean (*Lablab purpureus* L.) showed the order of increasing values on all the growth parameters under investigation. The highest seed germination percentage was recorded in T<sub>4</sub> treatment, with comparison of other treatments.

### Shoot length

The influence of biofertilizer and AMF fungi treatments on Lablab bean (*Lablab purpureus* L) of shoot length at 7, 15, 30, 60 and 90days were presented on Table 2. The maximum shoot length was observed on T<sub>4</sub> treatment (7.8, 19.6, 25.3, 34.7 and 46.3 cm/plant) respectively, when compare to other treatments

### Root length

The influence of biofertilizer and AMF fungi treatments on Lablab bean (*Lablab purpureus* L) of root length at 7, 15, 30, 60 and 90days were presented on Table 3. The maximum root length were observed on T<sub>4</sub> treatment (10.8, 20.3, 23.5, 32.0 and 37.6 cm/plant) respectively, with comparison of other treatments

### Total leaf area

The influence of biofertilizer and AMF fungi treatments on Lablab bean (*Lablab purpureus* L) of total leaf area at 7, 15,

30, 60 and 90days were presented on Table 4. The maximum total leaf area were observed on T<sub>4</sub> treatment (13.5, 20.3, 37.4, 41.5 and 47.5 cm<sup>2</sup>/plant) respectively, with comparison of other treatment

### Shoot fresh weight

The application of biofertilizer and AMF fungi treatments on Lablab bean (*Lablab purpureus* L) of shoot fresh weight at 7, 15, 30, 60 and 90days were presented on Table 5. The maximum shoot fresh weight were observed on T<sub>4</sub> treatment (750, 793, 842, 853, and 862 g/plant) respectively, with comparison of other treatment

### Shoot dry Weight

The application of biofertilizer and AMF fungi treatments on Lablab bean (*Lablab purpureus* L) of shoot dry weight at 7, 15, 30, 60 and 90days were presented on Table 6. The maximum shoot dry weight were observed on T<sub>4</sub> treatment (150, 182, 205, 216 and 253 g/plant) respectively, with comparison of other treatment.

### Root fresh weight

The influence of biofertilizer and AMF fungi treatments on Lablab bean (*Lablab purpureus* L) of root fresh weight at 7, 15, 30, 60 and 90days were presented on Table 7. The maximum of root fresh weight were observed on T<sub>4</sub> treatment (265.267, 270,278and289 g/plant) respectively, when compare other treatment.

### Root dry weight

The influence of biofertilizer and AMF fungi treatments on Lablab bean (*Lablab purpureus* L) of root dry weight at 7, 15, 30, 60 and 90days were presented on Table 8. The maximum of root dry weight were observed on T<sub>4</sub> treatment (10.3, 11.5, 12.5, 13.4 and 13.9 g/plant) respectively, when compare to other treatment.

Table 1. Germination percentage of sunflower

Sl. No.	Treatment	Germination (%)
1	T <sub>1</sub>	99.6
2	T <sub>2</sub>	99.5
3	T <sub>3</sub>	99.7
4	T <sub>4</sub>	100
5	T <sub>5</sub>	98.6

#### ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	2799.626	4	699.9064	282.7162	1.28E-14	3.006917
Columns	130.6216	4	32.6554	13.19064	6.13E-05	3.006917
Error	06.6104	16	2.47565			
Total	2969.858	24				

Table 2. Shoot length (cm/plant)

Treatments	7 DAS	15 DAS	30 DAS	60 DAS	90 DAS
T <sub>1</sub>	6.4	16.4	23.1	29.5	37.8
T <sub>2</sub>	6.0	15.5	22.5	28.4	35.5
T <sub>3</sub>	6.1	14.8	21.5	27.2	34.4
T <sub>4</sub>	7.8	19.6	25.3	34.7	45.3
T <sub>5</sub>	5.7	15.1	20.2	26.3	33.2

#### ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	2799.626	4	699.9064	282.7162	1.28E-14	3.006917
Columns	140.6216	4	32.6554	13.19064	6.13E-05	3.006917
Error	05.6104	16	2.47565			
Total	2969.858	24				

Table 3. Root length (cm/plant)

Treatments	7 DAS	15 DAS	30 DAS	60 DAS	90 DAS
T <sub>1</sub>	8.9	15.3	18.6	27.3	28.6
T <sub>2</sub>	8.7	16.1	17.9	26.4	27.9
T <sub>3</sub>	8.5	16.8	18.6	25.6	28.3
T <sub>4</sub>	10.8	20.3	23.5	32.6	37.6
T <sub>5</sub>	7.9	14.3	17.3	20.4	22.4

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	515.6815	3	171.8938	57.12182	2.24E-07	3.490295
Columns	202.873	4	50.71825	16.85412	7.25E-05	3.259167
Error	06.1411	12	3.00925			
Total	754.6655	19				

Table 4. Total leaf area (cm<sup>2</sup>/plant)

Treatments	7 DAS	15 DAS	30 DAS	60 DAS	90 DAS
T <sub>1</sub>	9.5	23.3	26.2	36.4	38.4
T <sub>2</sub>	8.9	21.2	25.2	34.9	37.4
T <sub>3</sub>	9.7	20.3	24.4	35.3	35.5
T <sub>4</sub>	13.5	29.3	37.4	41.5	47.5
T <sub>5</sub>	7.9	17.5	21.4	30.5	35.3

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	876.234	3	292.078	161.2503	5.84E-10	3.490295
Columns	369.42	4	92.355	50.9873	1.96E-07	3.259167
Error	21.736	12	1.811333			
Total	1267.39	19				

Table 5. Shoot fresh weight (g/plant)

Treatments	7 DAS	15 DAS	30 DAS	60 DAS	90 DAS
T <sub>1</sub>	665	775	783	787	867
T <sub>2</sub>	660	760	780	785	844
T <sub>3</sub>	657	780	781	784	850
T <sub>4</sub>	750	793	842	853	862
T <sub>5</sub>	650	762	769	771	794

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	13258.6	3	4419.533	16.95527	0.00013	3.490295
Columns	8587.3	4	2146.825	8.236165	0.001955	3.259167
Error	3127.9	12	260.6583			

Table 6. Shoot dry weight (g/plant)

Treatments	7 DAS	15 DAS	30 DAS	60 DAS	90 DAS
T <sub>1</sub>	128	175	188	198	242
T <sub>2</sub>	126	170	186	197	231
T <sub>3</sub>	125	179	190	195	241
T <sub>4</sub>	150	182	205	216	253
T <sub>5</sub>	123	155	163	175	184

## ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	9140.15	3	3046.717	48.09972	5.81E-07	3.490295
Columns	4357.5	4	1089.375	17.19839	6.56E-05	3.259167
Error	760.1	12	63.34167			
Total	14257.75	19				

Table 7. Root fresh (g/plant)

Treatments	7 DAS	15 DAS	30 DAS	60 DAS	90 DAS
T <sub>1</sub>	254	261	267	269	276
T <sub>2</sub>	252	260	266	268	274
T <sub>3</sub>	250	259	265	260	272
T <sub>4</sub>	265	267	270	278	289
T <sub>5</sub>	240	243	252	259	265

## ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	781.4	3	260.4667	40.53956	1.48E-06	3.490295
Columns	929.7	4	232.425	36.1751	1.32E-06	3.259167
Error	77.1	12	6.425			
Total	1788.2	19				

Table 8. Root dry weight (g/plant)

Treatments	7 DAS	15 DAS	30 DAS	60 DAS	90 DAS
T <sub>1</sub>	8.2	10.3	11.3	11.4	12.3
T <sub>2</sub>	8.9	10.1	11.2	11.0	12.2
T <sub>3</sub>	8.0	10.0	11.1	10.9	12.1
T <sub>4</sub>	10.3	11.5	12.5	13.4	13.9
T <sub>5</sub>	7.5	9.3	10.2	10.5	11.8

## ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	12.402	3	4.134	80.27184	3.28E-08	3.490295
Columns	12.57	4	3.1425	61.01942	7.11E-08	3.259167
Error	0.618	12	0.0515			

#### 4. DISCUSSION

The authors suggested that the biofertilizer help to microorganism in the faster decomposition of organic matter available in the soil, thereby increasing the availability of nutrients and ultimately in higher plant growth. These finding are in accordance with the findings of Mehta *et al.* (2010) in fenugreek, Thenmozhi (2010) in amaranth, in spinach, Meena *et al.* (2013) in fenugreek and Kavitha *et al.* (2013) in amaranth. Moreover, the inoculated AMF plant had a significant positive effect on all growth parameters regardless of single or mixed-species inoculation (Singh *et al.*, 2008; Cho *et al.*, 2009; Gogoi and Singh, 2011 and Ortas and Ustuner, 2014). However, (El- Khateeb *et al.*, 2010) which proved that bio fertilizers significantly increased the fresh and dry weights of roots. In contrast, the results disagrees with the study of Jnawali *et al.* (2015) which proved that combined application of bio-fertilizer with 50% of chemical fertilizers (N and P) has a positive role for safflower growth in comparison with chemical fertilizers alone. Inoculation of maize with AMF and BF have beneficial effect on supplying the plant with continuously available essential nutrients by which reflected on the plant growth and metabolism (Mazen *et al.*, 2018).

The dual inoculation of seed with Vesicular Arbuscular Mycorrhiza and Rhizobia enhanced the growth and quality traits i.e. protein and oil contents (Yadav and Ashok, 2015). Similarly Researches in the past few decades on various aspects of root symbionts have shown that dual interaction of AM fungi and Rhizobium has improved the growth, nodulation and yield (Talaat and Abdallah, 2008). According to Lokshman and Kadam (2011) that plants inoculated with both rhizobial and mycorrhizal symbiosis improved growth, nodulation and nitrogen fixation. Jarande *et al.* (2006) who stated that treatments had higher values of growth parameters including plant height, number of seeds per plant and pod length. Recent study suggest that the Effect of biofertilizer on the dry weight of plant was due to increased nitrogen uptake and the growth rate improvement. Effect of biostimulant on the dry weight of plant was due to increased nitrogen uptake (Kusuma *et al.*, 2019).

#### 5. CONCLUSION:

The study concluded that bio inoculant treatment is promising for *Lablab purpureus*; furthermore, bio inoculants are eco-friendly and inexpensive compared to chemical fertilizers. Inoculation of *Lablab purpureus* with AMF and biofertilizer have beneficial effect on supplying the plant with continuously available essential nutrients by which reflected on the plant growth and metabolism. This will reduce the needs for chemical fertilizers, and the kind and low quantity of chemical fertilizer have to be use will be to give the most satisfactory yields.

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