Effectiveness of Azospirillum culture on groundnut plants in a pot experiment

Yesha C. Patel, Sarvangi Rabara and Dr. Nutan Prakash

Department of M.Sc. Biotechnology;

Atmiya University

Pyesha310@gmail.com

Abstract:

The study of pot experiment of groundnut is use to determine the different physical characters and some nutrient availability test like; protein, proline, total sugar and the chlorophyll. Here uses particular region soil to isolate the azospirillum. And after screening we make soil, azospirillum culture and cocopeat containing mixture to grow the healthy and contamination groundnut seeds. Its take mainly 15- days to one month for growth so, during that periods water is provided appropriately take care of plants. Then perform the morphological characters test which contain plant height, Root length, shoot length, dry length, Fresh weight and number of roots, shoots and leaves and etc. And also perform nutrients availability test by different method. All test shows the remarkable result with azospirillum culture as compare to the other three plant sample. here control taken in sterile soil so it gives different result in praline stress condition and in other tests all nutrient amount is higher than control plant in samples. Therefore, to conclude that azospirillum culture is useful to make the biofertilizer.

1. INTRODUCTION

Increasing crop production to feed a growing population has driven the use of mineral fertilizers to ensure nutrients availability and fertility of agricultural soils. Fertilizers are food supplements for plants. In order to increase the soil's fertility and improve the yield and the production of nutrient-rich crops, fertilizers are added to the soil.

In general, these fertilizers can either be obtained naturally or artificially prepared for enhanced productivity of the yields. Biofertilizer are composed of natural materials that are extracted from animals, plants and vegetable waste materials on the other hand Chemical fertilizers or synthetic fertilizers are composed of non-organic and artificially cultivated elements. Biofertilizer are more advantageous than chemical fertilizers because farmers have complaining regarding chemical side effect and it is fulfilled with renewable source of biofertilizer which have numerical advantages like its make soil airy and biofertilizer are biodegradable, sustainable, and environmentally friendly. Bio fertilizers are usually prepared as carrier-based inoculants containing effective microorganisms. Incorporation of microorganisms in carrier material enables easy-handling, long-term storage and high effectiveness of bio fertilizers

Our aim to provide natural fertilizer to plants and here we were performed the pot experiment by taking Azospirillum culture which is mainly isolated from the Saurashtra region soil. Azospirillum is a Gram-negative, rod shape and nitrogen-fixing bacterial genus from the family of Rhodospirillaceae. That culture contains Acetobacter bacteria which has the ability to colonize the plant roots and fixing atmospheric Nitrogen. It is especially beneficial for sugarcane plantation as it activates the soil biologically and stimulates plant growth. Azospirillum culture makes the essential plant nutrients more available to plants. So, pot experiment is performed with the specific combination of: soil + bacterial culture + cocopeat. Regularly take care of pot plants is required to keep them healthy and infection free. In pot experiment, we took groundnut seed and let them grow in soil; which is sterile and add bacterial culture which is isolate from the

Saurashtra region soil. The temperature of the area required for groundnut should be around 27-30˚C for good germination and growth. It's required maximum one month for 10cm to 15cm growth and root and shoot elongation. The end of the experiment performs different test to check the physical characteristics and amount of nutrient present in the pot plant.

The purpose of this work was to produce Azospirillum cultures of high cell numbers suitable for production of inoculants that can sustain the shear and tear of the formulation process. This has been done by improving the performance of existing mass production culture media using available industrial sources.

2. MATERIALS AND METHODES

Here performing different test like morphological characters and check the nutrient availability by checking protein, proline, chlorophyll and total sugar in plant as compare to control to prove the Azospirillum culture as biofertilizer in mass production.

Firstly, for pot experiment there are mixture of the:

soil + bacterial culture + cocopeat + groundnut seeds

2.1 Method for physical characteristics

It is essential to check physical appearance after adding fertilizers to show improvement is soil physical conditions.

This method covers some characteristics like; **plant height, Root length, shoot length, dry length, Fresh weight and number of roots, shoots and leaves** for individual plants**.**

2.2 Method for knowing the nutrients present in each sample:

- estimation of chlorophyll, protein, sugar and proline

2.2.1 Estimation of chlorophyll

Chlorophyll is a green photosynthetic pigment which is helps plants to get energy from light. There may be many factors that affect the photosynthesis; the main factors are light intensity, carbon dioxide concentration, and temperature [1,2]. The chlorophyll content could depend on seasonal and environmental changes. The low chlorophyll a of phytoplankton observed during the winter; this may be affected from light limitation [3].

There are several methods to measure the content of chlorophyll, such as based on the absorption of light by aqueous acetone extracts of chlorophyll at laboratory [4,5], The solutions of 80% and 90% aqueous acetone (v/v) and 95% aqueous ethanol (v/v) were made from the analytical grade

acetone and ethanol and distilled water. The absorbance of chlorophyll solutions was measured spectrophotometrically (6) on 663nm and 645nm.

The formula is used for chlorophyll calculation is;

$$\textbf{Chlorophyll a} = \frac{\textbf{12.7} \times \textbf{A663} - \textbf{2.69} \times \textbf{A645} \times \textbf{v}}{\textbf{a} \times \textbf{1000} \times \textbf{w}}$$

$$\textbf{Chlorophyll b} = \frac{\textbf{22.9} \times \textbf{A645} - \textbf{4.68} \times \textbf{A663} \times \textbf{v}}{\textbf{a} \times \textbf{1000} \times \textbf{w}}$$

$$\textbf{total Chlorophyll} = \frac{(\textbf{12.7} \times \textbf{A663}) + (\textbf{22.9} \times \textbf{A645}) \times \textbf{v}}{\textbf{a} \times \textbf{1000} \times \textbf{w}}$$

where, a = length of light path in cell (1 cm), v = volume of extract in ml (final vol.) and w= fresh weight of sample in gram

by using this formula, we are able to know the total amount as well as chlorophyll a and b.

2.2.2 Estimation of protein

There are several studies done based upon analyzing the different, individual protein sources from various plant species are still on searching mode. The protein content was estimated by UV-Vis spectrophotometric technique using the conventional Lowry's method. Bovine Serum Albumin (BSA) was used as standard reagent against which unknown protein concentration of plants had been estimated.

Quantitative estimation of Proteins: in each of 1 ml extract, total protein content was estimated by the protocol of Lowry et al, 1951. Each sample, 5ml of freshly prepared alkaline solution (prepared by mixing 50 ml of 2% Na2CO3 in 0.1 N NaOH and 1 ml of 0.5% CuSO4. 5H2O in 1% sodium potassium tartrate) was added at room temperature and incubated for 10 minutes. Subsequently, to each of these mixture tubes 0.5 ml of Folin-Ciocalteureagent was rapidly added and incubated at room temperature (about 25oC) for 30 minutes until the blue color developed. The spintronic colorimeter was attuned at wavelength of 660 nm and set at 100% transmittance using blank before taking the readings of the standard and the test samples respectively.

Calculate with standard curve of protein i.e.

$$\textbf{y = 0.0744x + 0.0087, } \textbf{R}^2 \textbf{ = 0.9815}$$

2.2.3 Estimation of total sugar

Sugar Extraction:1gm of the chopped plant material was homogenized with 10 ml of 80% ethanol and then centrifuged at 10,000 rpm for 10 minutes. The pellet was discarded and the supernatant was used for further analysis (7).

Quantitative estimation of Reducing sugars: An aliquot from different extracts prepared for the estimation of total soluble sugar was used for the estimation of total reducing sugars according to the Nelson-Somogyi method. From the sample, a known volume of aliquot was pipetted out and was made up to 1 ml using distilled water. To this 1 ml of Somogyi's copper reagent was added. The mixture was then placed in boiling water bath for 20 minutes. After cooling under tap water 1 ml of Nelson's arsenomolybdate reagent was added with immediate mixing till the effervescence ceased (8). The intensity of color was measured after proper dilution at 540 nm by means of a Photochemical Digital Colorimeter.

By using sugar standard curve of sugar;

$$\mathbf{y = 0.0089 + 0.113,\ R^2 = 0.9957}$$

2.2.4 Estimation of proline

Salinity stress adversely affects productivity of cereal crops business worldwide and along with global climate change and population explosion is a risk for food security.

To perform proline test firstly take 0.5 g fresh leaf of groundnut in 2mL 3% sulfosalicylic acid, and then was filtered. 1mL filtrate with addition of 1mL acid ninhydrin and 1mL glacial acetic acid was heated in water Bath at 100◦ C for 1h. After being cooled at chilling temperature in ice bath ,2ml proline was added and vortexed for1 min, forming two layers. Above layer was taken and absorbance recorded with spectrophotometer at 520nm.

The formula for proline is:

$$\textbf{Proline content (mg. g}^{-1}\textbf{)} = \frac{\textbf{K value} \times \textbf{D.F} \times \textbf{Absorbance (O.D)}}{\textbf{Weight of sample}}$$

Where, O.D is at 520nm, Weight of sample = 0.5 gm, D.F is = 2.

3. RESULTS AND DISCUSSION

There are several tests which are performed to get best results of azospirillum culture as compare to the control plant.

3.1 Pot experiment results

So firstly, pot experiment was performed with three different Azospirillum species culture in triplicates.

These are image which show the results of the one control in triplicates and three different samples in triplicates.

Control:

Samples;

We can easily able to see the main difference in plant height both control and samples. So, by using azospirillum culture we can improve our pant growth than without culture soil in same time period i.e., 15 days tone month.

The images of sample 1, 2 and 3 is respectively shows the better effect and mainly sample 3 shows a greater number of leaves, branch and shoots as compare to the sample 1and 2.

3.2 Physical characteristics

After sample collection, fresh and dry weight of root and shoot, length of roots and shoots, length and width of Leaf along with leaf number per plant and number were recorded, and

Here this table shows the result of physical appearance of plant;

Method for physical characteristics

	Plant height (cm)	Root length (cm)	Shoot length (cm)	Dry length (gm)	Fresh weight (gm)	No. of roots	No. of shoots	No. of leaves
Control 1	11	3	8	0.11	0.39	5	7	30
Control 2	10	2	10			5	7	35
Control 3	10.5	2	7			7	6	40
Average	10.5	2.33	8.33	0.11	0.39	5.67	6.67	35
Sample 1- 1	14	5	7.5	0.29	0.52	7	8	50
2	12	5	11			10	9	52
3	14	6	10			9	8	46
Average	13.33	5.33	9.5	0.29	0.52	8.67	8.33	49.33
Sample 2 -1	15	7	6	0.26	0.46	11	7	49
2	14	6	11.5			9	6	34
3	14.5	6	7.5			8	9	55
Average	14.5	6.33	8.33	0.26	0.46	9.33	7.33	46
Sample 3- 1	16	7	10	0.28	0.55	10	9	38
2	13	5	11.5			9	10	62
3	15.5	5.5	10			10	7	39
Average	14.83	5.83	10.5	0.28	0.55	9.67	8.67	46.33

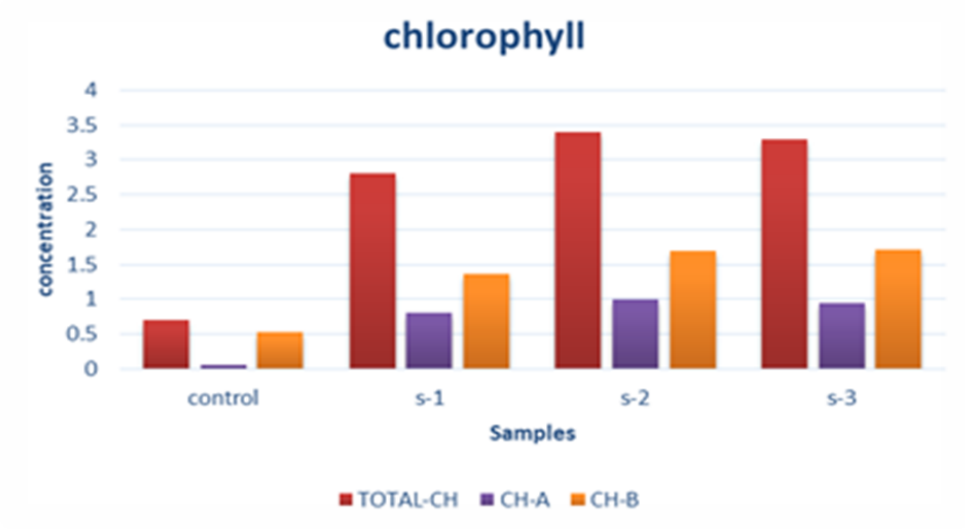
3.3 Method for knowing nutrient availability in plants:

3.3.1 Result of estimation of chlorophyll

This graph is representing the amount of chlorophyll A, chlorophyll B and total chlorophyll;

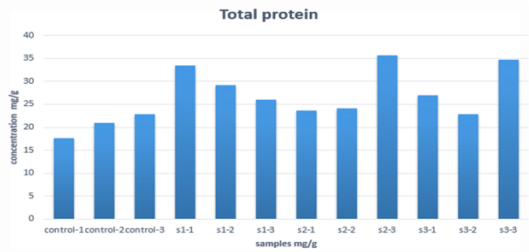
This bar graph showed the result of total chlorophyll, chlorophyll a and chlorophyll b in all three samples and the plants of control.

The amount of chlorophyll a and chlorophyll b combine give the amount of the total chlorophyll present in the pot plants.



3.3.2 Estimation of protein

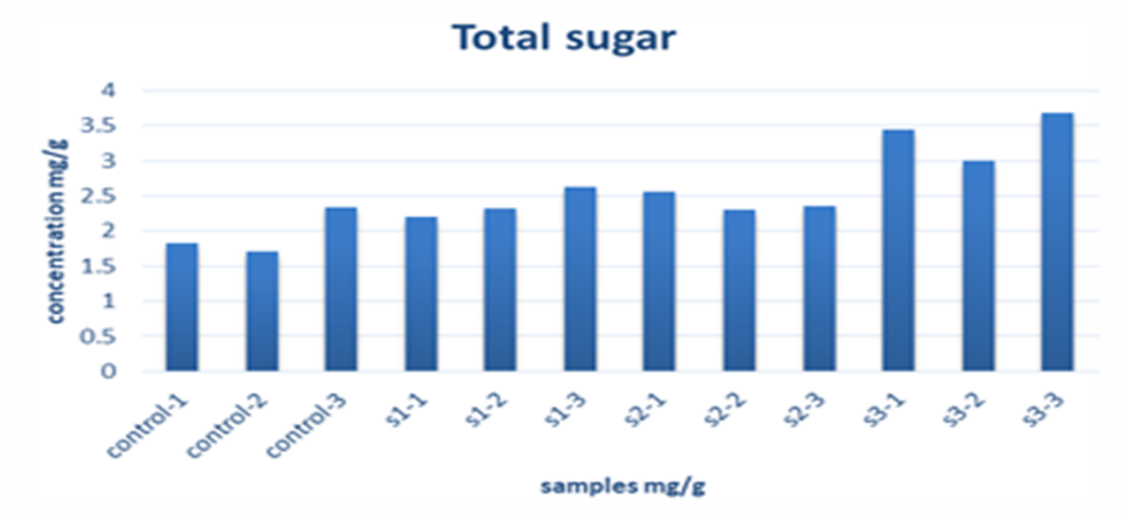
This graph is representing the total amount of protein present in the control and samples;



This graph is describing the more amount of protein present in the three samples as compare to the controle plants in all triplicate pot experiments.

3.3.3 Results of estimation of total sugar

This graph is representing the total amount of sugar present in the control and samples;

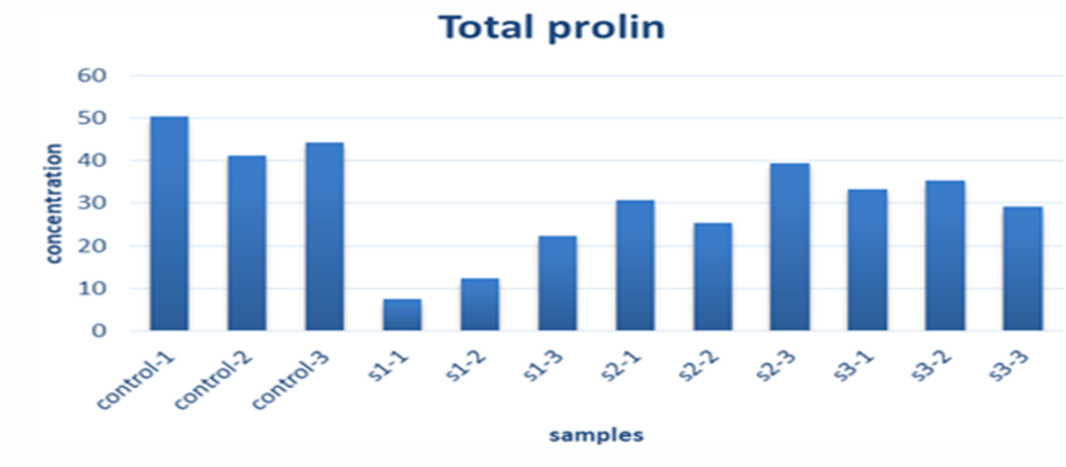


This graph is representing the total amount of sugar present in the plant leaves but it does not show the amount of the total carbohydrates. so, there is more amount present in the sample 3 as compare to the sample 1and 2 as well as controle.

3.3.4 Result of estimation of proline

This graph is showing the result of the amino acid; proline amount presents in controls and samples.

It shows the highest value in the all three control plants as compare to the three samples, because there no stress condition in all three sample. The soil is sterile and it show the different between normal soil which is use to grow the all three control plants.



So, it's easy to determine that there is no stress in all sample plant but there is stress in all control because normal soil contain some salts in it in a high amount, which are responsible to give stress to plant.

Sample plants are not suffered with the any stress condition because there is sterile soil use for them and it is also helpful to determine that; azospirillum culture has capacity to grow plant stress free.

4. CONCLUSION

To conclude that, there is no such side effect in any plant after using azospirillum culture in pot experiment.so, this culture is safe to use in future as a biofertilizer.

Pot result shows the visible difference between the samples and control; there in no yellow leaves in plant and no other deficiency is there, and mostly all physical characters are high in sample plants then control plant like: height, number of roots, shoots .leaves etc.

This experiment can help people by letting them know which soil is better to grow plant easily. And it shows the markable difference between with azospirillum culture plants and without. Azospirillum culture also shows the effective results, and it shows the good combine capacity with soil and cocopeat.

Cocopeat goes correctly with soil and azospirillum culture mixture and its make results more differentiable. Without use of cocopeat plants can may be not get proper airy soil in root region to get the oxygen.

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