A Comparative Study of Efficiency of Bio-Inoculants (*VAM, Trichoderma, Azospirillum and PBS*) On Crop Plants (*P. Vulgaris, Zeamays, Arachis hypogaea and Eleusine coracana).*

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Abstract:

A comparative study of Bio-inoculants was carried out under laboratory condition. In this study we took plants (*P. vulgaris, Zeamays, Arachis hypogaea and Eleusine*) as control and test sample. VAM, tricoderma were mixed in the soil for test sample separately and irrigated once in a day for 15 days. After 15 days the leaf extracts of each individual set of test and control sample were taken and analysed and estimated for protein, glucose and pigment chlorophyll a using lowry's method, antheron method and double beam uv spectrometry respectively. At this basis we could found the large difference between test and controlled plant contents. And we conclude that if we use bio-fertilizer such as VAM, *Trichoderma, Azospirillum*, and PSB all together, we can get 200-500 % more yield of protein, 300-1000 % yields of glucose and 10 times more chlorophyll a.

Keywords:
Vessicular Arbuscular Mycorhhiza, Trichoderma, Azospirillum Phosphate solubilizing bacteria.

Introduction:

A bio-fertilizer (also *bioinoculants*) is a substance which contains living microorganisms which, when applied to seed, plant surfaces, or soil, colonizes therhizosphere or the interior of the plant and promotes growth by increasing the supply or availability of primary nutrients to the host plant. Bio-fertilizers add nutrients through the natural processes of nitrogen fixation, solubilizing phosphorus, and stimulating plant growth through the synthesis of growth-promoting substances(G. A. Khan 2007). Bio-fertilizers can be expected to reduce the use of chemical fertilizers and pesticides(S. Kanazawa 1988). The microorganisms in bio-fertilizers restore the soil's natural nutrient cycle and build soil organic matter. Through the use of bio-fertilizers, healthy plants can be grown, while enhancing the sustainability and the health of the soil(S. Kanazawa1988). Since they play several roles, a preferred scientific term for such beneficial bacteria is "plant-growth promoting *Rhizobacteria*" (PGPR).

Therefore, they are extremely advantageous in enriching soil fertility and fulfilling plant nutrient requirements by supplying the organic nutrients through microorganism and their by-products(J.I. Baldani2002).Hence, bio-fertilizers do not contain any chemicals which are harmful to the living soil.

Bio-fertilizers areeco-friendly organic agro-input and more cost-effective than chemical fertilizers. Bio-fertilizers such as Rhizobium, *Azotobacter*, *Azospirillum* and blue green algae (BGA) have been in use a long time(S.W. Cline 1989). *Rhizobium* inoculant is used for leguminous crops. *Azotobacter* can be used with crops like wheat, maize, mustard, cotton, potato and other vegetable crops. *Azospirillum* inoculations are recommended mainly for sorghum, millets, maize, sugarcane and wheat. *Anabaena* in association with water fern Azolla contributes nitrogen up to 60 kg/ha/season and also enriches soils with organic matter. Other types of bacteria, so-called phosphate-solubilizing bacteria, such as *Pantoeaag glomerans* strain P5 or *Pseudomonas putida* strain P13, are able to solubilize the insoluble phosphate from organic and inorganic phosphate sources. In fact, due to immobilization of phosphate by mineral ions such as $Fe^{++/+++}$, Al^{++} and Ca^{++} or organic acids, the rate of available phosphate (Pi) in soil is well below plant needs. In addition, chemical Pi fertilizers are also immobilized in the soil, immediately, so that less than 20 per cent of added fertilizer is absorbed 2 by plants(R.A. Lawley 1983; S.W. Cline 1989). Therefore, reduction in Pi resources, on one hand, and environmental pollutions resulting from both production and applications of chemical Pi fertilizer, on the other hand, have already demanded the use of new generation of phosphate fertilizers globally known as phosphate-solubilizing bacteria or phosphate bio-fertilizers. A bio-fertilizer provides the following benefits: 1. The Function of biofertilizer is to improve soil fertility. It maintains the natural habitat of the soil. It increases crop yield by 20-30%, replaces chemical nitrogen and phosphorus by 25%, and stimulates plant growth. It can also provide protection against drought and some soil-borne diseases.

Material and method:

Glassware's and Chemicals, Samples, Fields, oil, Plants, Biofertilizers:

Measuring cylinder, test tube, glass rod, beaker, were used.10 ml centrifugation tubes, micropipette, waterbath, Centrifuge machine, Spectrophotometer, mortor and pestle, knife, distillation unit, Leaves of plants such as groundnut, kidney bean, ragi, maize which treated with biofertilizers such as Trichoderma, Azospirillum, PSB, and VAM. Plants such as Kidney bean, maize, groundnut, raggi Concentration of biofertilizers: 25 gm, 50gm, 75gm, 100gmBiofertilizers: Trichoderma, Azospirillum, Vesicular Arbuscular Mycorrhiza, Phosphate Solubilizing Bacteria. 68 bags filled with soil and biofertilizers.

1. **Extraction and estimation of proteins by Lowry's method–**

Requirements Reagent (A) -0.5 % copper sulphate in 1% potassium sodium tartarate in distilled water and this should be prepared fresh. Reagent (B) – 2% sodium carbonate in 0.1N NaOH. Reagent(C) –Alkaline Copper Reagent – mix

50 ml of copper reagent (A) and (B) 1ml just before use.Test Sample – 3gm of leaflet can be used. Standard Protein Solution – 0.02 gm of protein (BSA) is dissolved in 100 ml of dist. Water which give 200 µg/ml of concentration. Fc reagent – 1N FC reagent is used for estimation. Glasswares–Test tubes, pipette, centrifuge tube, conical flask, standard volumetric flask, mortar and pestle, colorimeter. Procedure– Sample prerparation –3 gm of fresh leaflets homogenized with 10 PBS (phosphate buffer saline) and centrifuge at 3000-5000 rpm for 10 min. supernatant is used as source of protein estimation.

Estimation of protein:

Preparation of standard graph: Different aliquots of standard protein solution (200 mg/ml) ranging from 0.2 to 1 ml were pipette out in to different test tube. The volume of each test tube made up to 1ml using distilled water.5.0 ml of alkaline copper reagent was added to all tubes mix and allow to stand for room temperature in order to dissolve the protein. To this 5.0 ml of FC reagent 1:1 dilution were added and mixed thoroughly. The tubes were allowed to stand for 30 min at room temperature. The absorbance was read at 660 nm against the suitable blank.The graph of concentration of protein in µg on x-axis and optical density on y-axis are plotted. The amount of protein in test sample is calculated from the standard graph.

Preparation of biological sample: 1gm of sample like kidney bean, maize, groundnut, raggi leaf after 15 days which were treating with biofertilizers was homogenized in 10 ml of freshly prepared 1% NACl. It was then centrifuged at 3000 rpm for 5-8 minutes.To the supernatant containing the protein 10 ml of 10% TCA was added. Shaken well and centrifuged at 3000 rpm for 5-8 minutes. The precipitate obtained was dissolved in 1N NaOH and the volume was made upto10ml of 0.1 N NaOH. This secured as a biological sample. Take 0.1 ml of biological sample and add 0.9 ml of 1N NaOH and make volume 1 ml. After that add 5 ml of reagent C and incubate at room temperature for 20 minutes. After that add 1ml of FC reagent and again incubate at room temperature for 20 minutes and take OD value by using calorimeter at absorbance 660 nm. Plot the point on the standard graph and take a value on x axis of concentration of protein. Calculate the concentration of protein by using graph value.

2. Extraction and estimation of glucose by Anthrone reagent method

Requirements - Anthrone Reagent- Dissolve 200 mg of anthrone in 100 ml of ice cold 95% H2SO4 prepare fresh and 0.5 N HCl. Standard Glucose Solution -dissolve 200mg of glucose in 100 ml of distilled water. Working Solution - 10 ml of stock is diluted by 100 ml of dist. Water to obtain 200µg/ml glucose standards ,the stock can be stored in refrigerator after adding few drops of toluene. Other requirements-centrifuge, centrifuge tube,

boiling water bath, colorimeter. Biological Sample-1 gm of fresh leaflets of Kidney bean, groundnut, maize, raggi.

Estimation of glucose: Preparation of standard graph: Different dilutions ranging from 20-200 µgml-1ranging from 0.2 to 1.0 ml were pipette out in to different test tube and the volume was made up to 1 ml with distilled water.3 ml of anthrone reagent was added to each tube and the tubes are kept in boiling water both for 15 minutes for colour development. The tubes are cooled and OD was measured at 650 nm.

Preparation of biological Sample: Weighed 1 gm of biological sample and homogenised in 2 ml of 0.5 N HCl and 7 ml of distilled water was added which gives 10% homogenate. The homogenate was centrifuged at 3000 rpm for 10 minutes. The supernatant was used for the estimation of total soluble sugar after recording the volume of supernatant.0.01 ml of supernatant was taken and made up to 1 ml using distilled water. The volume was made up to 1 ml in all the tubes including the sample tubes using distilled water. 3 ml of anthrone reagent was added (freshly prepared) and the tubes were kept in boiling water bath for 15 minutes. The tubes were cooled to room temperature and absorbance was read at 630 nm using blank. A standard graph was drawn and the amount of total sugar present in the given sample was noted.

3. Extraction and estimation of pigment chlorophyll 'a' & 'chlorophyll 'b'

Requirements – Leaf 5 gm, isolation medium (PBS) pestle and mortar cheese cloth and beaker centrifuge tube, spectroscopy photometer.

Procedure: 1 gm of fresh green leaves was finely cut into pieces and taken in a pre-chilled pestle and mortar maintained in ice bath. The tissue was homogenised into smooth paste in 80% chilled acetone containing a pinch of Mg carbonate (MgCO3). During homogenation care should be taken to ensure homogenate should not be allowed to intense light and prevented from drying wash the homogenate repeatedly until all the washings are colourless completely. Washings are then cooled and centrifuge at 5000 rpm for 8 minutes at 4OC. All operations are carried out in dim light at 4OC. The total volume of extract is recorded. If the green colour is too intense it may be appropriately diluted using 80% acetone and the dilution factor is noted. The absorbance is read in spectrophotometer at 663 nm and 645 nm using 80% acetone as blank. In spectrophotometer absorbance of chlorophyll is read at 645 and 663 nm by Arnon formula (1948) is used for the calculations of chlorophylls a and b.

Arnon's Formula:

1)MgChl _a' g/l of fresh leaf = { 12.7[A633]-2.69[A645]}× V/1000×w

2)MgChl'b' g/l of fresh leaf = { 22.9[A645]-4.68[A663]}× V/1000×w

3) Total Chl Mg g/l = { 20.2[A645]+8.02[A663]}× V/1000×w

Where, A = absorbance of specific nm, V = volume of Chlorophyll extract (final)

w = fresh weight of the tissue in green leaf

Result and discussion:

After graph calculation, following results were found. There is a comparative result between control and biofertilizers. Biofertilizers increasing concentration of protein, sugar and chlorophyll as compare to control plants.

3.Chlorophyll content in plants

Different biological samples like kidney gram, maize groundnut, ragi leaves are collected and the amount of Chlorophyll 'a', 'b' and total chlorophyll is mg/g was calculated by using Arnon formula and the results are tabulated as follows:

Conclusion:

As per result, biofertilizers results, there is increased in mineral and water uptake, root development, vegetative growth and nitrogen fixation. Some Biofertilizers such as *Trichoderma***,** *Azospirillum* sp. stimulate production of growth promoting substance like vitamin-B complex, Indole acetic acid (IAA) and Gibberellic acids etc, so that plants were increasing faster than normal plants such as maize, kidney gram, raggi, and groundnut. PhosphatemobilizingorphosphorussolubilizingBiofertilizers/microorganisms (bacteria, fungi, mycorrhiza etc.) convert insoluble soil phosphate into soluble forms by secreting several organic acids and under optimum conditions they can solubilize/mobilize soil due to which crop yield may increase. Mycorrhiza or Vesicular Arbuscular Mycorrhizae (VAM) fungi is often used as biofertilizer. VAM provides significant amount of nutrients to the plants such as copper, zinc, phosphorus and sulphur by making their widely extended hyphal network on the upper or lower side of the soil layer. VAM is commercially used in the fields of India when used as Biofertilizers enhance uptake of P, Zn, S and water, leading to uniform crop growth and increased yield and also enhance resistance to root diseases and improve hardiness of transplant stock. They liberate growth promoting substances and vitamins and help to maintain soil fertility. They act as antagonists and suppress the incidence of soil borne plant pathogens and thus, help in the bio-control of diseases, Nitrogen fixation. Phosphate mobilizing and cellulolytic microorganisms in bio-fertilizer enhance the availability of plant nutrients in the soil and thus, sustain the agricultural production and farming system. They are cheaper, pollution free and renewable energy sources .They improves physical properties of soil, soil tilts and soil health in general.

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Paper Submission I 10th April 2015: submission.icsts2015@gmail.com
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