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# PRODUCTION OF BIOFERTILIZER FROM PHOSPHATE SOLUBILIZING FUNGI

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**Abstract**: Phosphorus is present in plant and animal cells. It is vital to all plants for harvesting the sun's energy and converting it into growth and reproduction. (PSMs) are a group of beneficial microorganisms capable of hydrolyzing organic and inorganic insoluble phosphorus compounds to soluble P form that can easily be assimilated by plants. (LBF) are suspensions having useful microorganisms, which solubilize insoluble phosphates and make it available for the plants. Pikovskaya's medium is recommended for cultivation of phosphate solubilizing microorganisms. PSF were confirmed on Pikovskaya's agar plate by formation of halo zones around the fungal colonies and were further determined to be mucor using lactophenol blue staining. Pure and mixed cultures were used for preparing 100% and 50% concentrations respectively. Different concentrations of 100%, 75%, 50% and 25% of colonies with halo zones were prepared and mixed with cow dung compost in 1:1 ratio to check its efficacy as a liquid biofertilizer with moong plant. Highest growth was observed with 75% and lowest with 25% while other concentrations showing moderate growth.

Keywords: PSMs, LBF, Pikovskaya's agar, lactophenol blue staining, halo zones, moong plant.

## I. INTRODUCTION

Phosphorus is an essential nutrient both as a part of several key plant structure compounds and as a catalysis in the conversion of numerous key biochemical reactions in plants. Phosphorus is noted especially for its role in capturing and converting the sun's energy into useful plant compounds. Phosphorus is a vital component of DNA, the genetic "memory unit" of all living things. It is also a component of RNA, the compound that reads the DNA genetic code to build proteins and other compounds essential for plant structure, seed yield and genetic transfer. The structures of both DNA and RNA are linked together by phosphorus bonds. Phosphorus is a vital component of ATP, the "energy unit" of plants. ATP forms during photosynthesis, has phosphorus in its structure, and processes from the beginning of seedling growth through to the formation of grain and maturity. Thus, phosphorus is essential for the general health and vigor of all plants. The total phosphorus content of most surface soils is low, averaging only 0.6% phosphorus. This compares to an average soil content of 0.14%

nitrogen and 0.83% potassium. Soil P is classified into inorganic and organic P, and 60–80% of the total P is inorganic P, since P is an element of the sedimentary cycle. According to the P fractionation method provided by Chang and Jackson (1957), the inorganic P was divided into five main groups including soluble P, calcium phosphate, aluminum phosphate, iron phosphate and occluded P. Generally, the soluble P, which can be directly absorbed by plants, accounts for a little proportion in soil, while the rest of inorganic P, such as calcium phosphate, aluminum phosphate, iron phosphate exist with hard-to-dissolve forms. Phosphate-solubilizing microorganisms are recognized as a solution to the challenges in P fertilization management due to their abilities to mobilize P from recalcitrant sources (Yang Zhang et al., 2018).

Fungi are widely distributed across terrestrial, marine, and freshwater environments. Fungi play important roles in both economics and ecology as sources of food and medicine that also provide decomposition services. Moreover, fungal research can lead to breakthroughs in microbial biotechnology and other industries (Mingkwan Doilom et al., 2020). Phosphate-solubilizing fungi (PSF) are able to enhance the solubilization of insoluble phosphate (P) compounds. PSM employs the following three mechanisms (McGill and Cole 1981) to solubilize P: (a) By releasing compounds such as hydroxyl ions, protons, siderophores, organic acids, and CO2 that assist the breakdown and solubilization of complex molecules.(b) Biochemical mineralization by the discharge of extracellular enzymes. And (c) By releasing phosphorous during substrate degradation.

PSMs produce organic acids such as oxalic, fumaric, glyoxalic, malic, citric, gluconic, succinic, alpha-ketobutyric, 2-ketogluconic, and tartaric acid which lower the pH (Puente et al. 2004; Rodrigues et al. 2004). Its amount and type vary from fungus to fungus. Lowering of pH of the fltrates of PSMs is because of these organic acids (Rani et al. 2013). Fasim et al. (2002) observed the role of microbes in the solubilization of zinc oxide and phosphate through gluconic acid and 2-ketogluconic acid production. Proton and enzyme theory states that a group of enzymes such as esterase are responsible for the phosphorous solubilization from compounds containing organic phosphate. According to this theory, phosphorous solubilization, besides the generation of acid, involves release of protons in association with ammonium assimilation (Shahab et al. 2009). Other than these two systems, phytohormones such as indole acetic acid, cytokinin, and gibberellin also aid phosphate solubilization. Formation of chelating agents such as H2S, CO2, mineral acids, and siderophores also has indirect effect on phosphate solubilization (Shahab et al. 2009).

Biofertilizer can be defined as biological products containing living microorganisms that, when applied to seed, plant surfaces, or soil, promote growth by several mechanisms such as increasing the supply of nutrients, increasing root biomass or root area and increasing nutrient uptake capacity of the plant (Vessey, 2003). Some of the advantages associated with biofertilizers include: • They are eco- friendly as well as cost effective • Their use leads to soil enrichment and the quality of the soil improves with time. • Though they do not show immediate results, but the results shown over time are spectacular. • They increase the phosphorous content of the soil by solubilising and releasing unavailable phosphorous. • Biofertilizers improve root proliferation due to the release of growth promoting hormones. • Microorganism converts complex nutrients into simple nutrients for the availability of the plants (Rakesh Kumar et al. 2017)

Microbiology may offer sustainable solutions to mitigate the problems of plant P nutrition in the light of the finite, non-renewable nature of P fertilizers. The object of this study was to screen and isolate microorganisms from soil that can solubilize phosphate and production of liquid biofertilizer for the enhancement of plant growth.

## II. LITERATURE SURVEY

Phosphorus (P) is one of the major bioelements limiting agricultural production. Phosphate solubilizing fungi play a noteworthy role in increasing the bioavailability of soil phosphates for plants. The study was aimed at isolating and characterizing phosphate solubilizing fungi from different rhizospheres. A total of 359 fungal isolates were obtained from 150 rhizosphere soil samples of haricot bean, faba bean, cabbage, tomato, and sugarcane. Among the isolates, 167 (46.52%) solubilized inorganic phosphate. The isolated phosphate

solubilizing fungi belonged to genera *of Aspergillus (55.69%), Penicillium spp. (23.35%), and Fusarium (9.58%).* Solubilization index (SI) ranged from 1.10 to 3.05. Isolates designated as JUHbF95 (*Aspergillus sp.)* and JUFbF59 *(Penicillium sp*.) solubilized maximum amount of P 728.77 $\mu$g·mL−1 and 514.44 $\mu$g mL−1, respectively, from TCP (tricalcium phosphate) after 15 days of incubation (Firew Elias et al, 2016).

Phosphate-Solubilizing Fungi and Alkaline Phosphatase Trigger the P Solubilization during the Co-composting of Sorghum Straw Residues with Burkina Faso Phosphate Rock was studied on increase the nutrient bioavailability of PR-enriched composts by inoculations with phosphate-solubilizing microorganisms. Their higher nutrient content makes these PR-enriched composts more efficient for plant growth, and they are more environmentally safe. PR-enriched composts with rhizosphere soil and the mechanisms that sustain it. Elucidating the P solubilization mechanisms is an essential step forward in the fabrication of better-quality composts with higher available P concentrations (Papa Saliou Sarr et al, 2020).

Liquid biofertilizer (LBF) technology can be considered as a breakthrough in the field of biofertilizer technology as it rectifies all the drawbacks of the carrier based biofertilizers. With these benefits, the technology should find greater acceptances, extension workers and commercial biofertilizer manufacturers the increased adoption of this technology in Indian agriculture in years to come (Hegde, 2002).

Phosphorus (P) is a macronutrient required for the proper functioning of plants. Because P plays a vital role in every aspect of plant growth and development, deficiencies can reduce plant growth and development. Though soil possesses total P in the form of organic and inorganic compounds, most of them remain inactive and thus unavailable to plants. Since many farmers cannot afford to use P fertilizers to reduce P deficits, alternative techniques to provide P are needed. Though PSMs have been a subject of research for decades, manipulation of PSMs for making use of increasing fixed P in the soil and improving crop production at the field level has not yet been adequately commercialized. The purpose of this review is to widen the understanding of the role of PSMs in crop production as biofertilizers. (GirmayKalayu et al, 2019).

In this research five low cost liquid formulations were examined. Formulations 1, 2 and 3, were phosphate buffer, 0.2% and 0.5% KNO3 dissolved in phosphate buffer, respectively. Formulation 4 was nutrient broth containing 4% glycerol and formulation 5 was diluted nutrient broth containing 4% glycerol. Survival (cfu) and phosphate solubilization index (SI) were evaluated after 3 months. *Pseudomonas putida strain P13&and Pantoeaagglomerans strain P5* were selected. Considering strain P5, increase in KNO3 concentration decreased preserving ability. Overall, less nutritious formulations (1 and 5) provided maximum preserving ability without bioactivity loss. In the case of strain P13, maximum survival obtained in formulations 2 and 3, whereas SI level decreased. Preserving ability in formulations 1, 4 and 5 was similar but less nutritious formulations (1 and 5), improved bioactivity. The results introduced two formulations of 1 and 5 as economically efficient liquid bio inoculants for *Pseudomonas putida and Pantoea agglomerans.* (Sanaz Goljanian-Tabrizi et al, 2016).

Same type of research was carried out by (N.Mounika1 *et al***)** Effect of different Chemical Additives on growth of *Azotobacter vinelandii*. In this experiment, we evaluated different concentrations of three Different chemical amendments viz, polyvinyl pyrrolidone (PVP), gum arabica, and sodium alginate for their ability to support growth and promote survival of *Azotobacter vinelandii* in nutrient broth during the storage. *Azotobacter vinelandii* inoculated with PVP (4%) promoted highest cell population followed by gum arabica (0.5%) and sodium alginate (0.2%). The results of the present study clearly indicated that the 4% PVP probably gave higher cell population than other additives.

But Similarly The liquid formulation of efficient PGPR was prepared in sterilized liquid manure matkhakhaad with the addition of four protective substances glycerol (2%), polyethylene glycol [PEG – 400 1%], polyvinylpyrrolidone-30 [PVP 1 %], and trehalose (1%), and their capacities for maintaining cell viability during storage in low, medium, and high temperature and pH ranges were evaluated. Trehalose (1 %) was chosen as a potential additive because it could maintain a relatively high population and conferred greater microbial vitality under various storage conditions matkhakhaad is considered a safe, low-cost, and easy-to-

process material, and this formulation would facilitate the practical use of PGPR in agriculture.(Manimekalai G and Kannahi M *et al* 2018).

The study consists of Screening of Phosphate-Solubilizing Fungi from Air and Soil in Yunnan, China: Four Novel Species in *Aspergillus, Gongronella, Penicillium, and Talaromyce.* These fungal strains were tested for their ability to solubilize tricalcium phosphate (TCP) on both solid and liquid Pikovskaya (PVK) media in vitro. The airborne fungal strain KUMCC 18-0196 (*Aspergillus hydei sp. nov*.) showed the most significant phosphate solubilizing activity on a solid PVK medium with the solubilization index (SI) (2.58 ± 0.04 cm) and the highest solubilized phosphates (1523.33 ± 47.87 μg/mL) on a liquid PVK medium. *(*Mingkwan Doilom *et al).*

Phosphate-solubilizing microorganisms (PSM), especially plant growth-promoting fungal stains (PGPF), have the potential to solubilize insoluble P to soluble forms through chelation and ion exchange processes, organic acid production, thus making phosphorous available to plants. Therefore, the use of phosphate-solubilizing fungi (PSF) along with RP is considered to be a cost-effective means for facilitating the P availability. Application of biofertilizers reduces the adverse effects of chemical fertilizers on the health of plant vis-à-vis the fertility of the soil (Gurdeep Kaur *et al*, 2017).

(Adekunle Raimi et al), in their study emphasized on underdevelopment of biofertilizer industry in many African countries. The findings show that inadequate biofertilizer research, lack of technology development, and ineffective regulatory framework have largely contributed to the challenges of biofertilizer development in Africa. To achieve increased commercial production and optimal application of biofertilizer amongst farmers in Africa, Adequate and effective extension programs, agro market development, as well as agricultural and research institution development, could improve the production and adoption of biofertilizers in Africa is needed .

(Wenkai Duan et al), in their study tested whether the biofertilizer Saccharomyces cerevisiae can enhance the aroma of strawberry. Mycorrhizal fertilization (MF) and foliar fertilization (FF), to investigate the effects of S. cerevisiae on various characteristics of F. ananassa. The results showed that the application of yeast under MF significantly increased the amount of soluble sugars and total volatiles. However, no significant difference was detected in the anthocyanin content, the amount of total volatiles in fruits under different treatments was 18.17 (MF), 11.78 (FF), and 9.51 (control) lg and 1 fresh weight (FW). The main volatiles obtained from the fruits under MF, FF, and control treatments were esters (51.45%, 44.39%, and 29.39%, respectively), alcohols (35.93%, 28.77%, and 6.58%, respectively), and aldehydes (5.86%, 18.31%, and 62.88%, respectively), the photosynthetic rate and intercellular CO2 concentration were significantly influenced by the utilization of yeast strains. The MF treatment with yeast resulted in higher photosynthetic rate, and the plants from the FF treatment recording the highest intercellular CO2 concentration. S. cerevisiae, by generating CO 2, promotes photosynthesis, leading to the increased sugar con-tent in the fruits, which subsequently enhances the content of 3(2H)-Furanone, 4-methoxy-2, 5-dimethyl (DMMF) and phenylalanine-derived volatiles.

(Deepali Chittora et al). In their study explored the most ecofriendly approach ‘green technology technique for biofertilizer preparation. Cyanobacteria are emerging candidates for efficiently conversion of radiant energy into chemical energy. This biological system produces oxygen as a by-product. Cyanobacterial biomass can also be used for the large scale production of food, energy, biofertilizers, secondary metabolites, cosmetics and medicines.

## III. METHODOLOGY

- **SAMPLE COLLECTION**

Twenty grams of soil sample was collected from a mild hot region of Mumbai.

- **ENRICHMENT OF SOIL SAMPLE**

The collected soil sample were separated from other impurities. Soil sample were further enriched in pikovasky's broth medium containing (g/L): 0.5 g (NH4)2SO4, 0.1 g MgSO4•7H2O, 0.02 g NaCl, 0.02 g KCl, 0.003 g FeSO4•7H2O, 0.003 g MnSO4•H2O, 5 g Ca3 (PO4)2, 10.0 g glucose, 0.5 g yeast extract, and 1000 mL distilled water. The medium was autoclaved at 121°C for 15 minutes.1g of soil sample was inoculated in 50 ml of pikovasky's broth medium and incubated for 7 days at room temperature in shaker condition.

- **ISOLATION OF PHOSPHATE SOLUBIZING FUNGI**

Isolation of phosphate solubilizing fungi was done on pikovasky's agar medium. A loop from enrichment broth was streaked on sterile pikovasky's agar medium and incubated at room temperature for 2 to 7 days. After incubation plates were screened for fungal colonies showing clear zones around the colonies. The colonies were further sub cultured on sabouraud dextrose agar slant at 4∘ C for further investigation.

- **IDENTIFICATION OF CHARACTERISTICS OF ISOLATES**

After isolation on pikovasky's agar medium colony characteristics were determined of each isolate showing halo zones. Isolated showing white colonies with halo zones were identified by performing fungal staining using lactophenol cotton blue dye. On a clean glass slide one or two drops of lactophenol blue was added. A mycelial mat was transfer on fluid was a sterile needle and pressed gently so it mixes with stain. Fungal mycelia was spread on the slide carefully making a thin preparation and coverslip was placed and observed under microscope.

- **PREPARATION OF BIOFERTILIZER**

Pure culture isolates were inoculated in pikovasky's broth medium for 7 days at room temperature in shaker condition. After 7 days of incubation fungal growth in conical flask was mixed with media by shaking vigorously. Isolate 1 and isolate 2 were diluted using sterile distilled water. Different concentration were prepared using sterile distilled water as 100%, 75%, 50%, 25%. Also isolate 1 and isolate 2 were mixed together and used at concentration of 100% and 50%.

All concentrations of PSF were mixed with a cow dung compost acting as a carrier component in 1:1 ratio. Carrier materials were passed through a 100-mesh sieve before The main criteria used to select carrier materials were the ability to adjust the pH to neutral (pH 7.0), high water holding capacity, low cost, and wide availability. Thus biofertilizer was ready to use for application.

- **POT PREPARATION**

Garden soil was bought from an area near Mumbai. Soil was clean from other impurities such as dried leaves or other unwanted particles. Soil was mixed with cocopit at 1:1 ratio. The mixture was transferred into 7 pots which were 12 cm each.

- **TESTING OF BIOFERTILIZER**

Moong seeds were selected on the basis of their property to germinate faster than other. Moong beans are tightly packed therefore they are forced to germinate sooner and thicker. 10 moong seeds were soaked in each different concentration of biofertilizer. After an hour of soaking the seeds with biofertilizer were sowed in the soil mixture at 2cm depth. Seeds were watered daily on time at morning. Plant was evaluated after 7 days for its growth difference in different concentrations.

## IV. RESULTS AND DISCUSSION

### ➢ ENRICHMENT OF PHOSPHATE SOLUBILIZING FUNGI

Soil sample was inoculated in pikovasky's broth medium for 7 days at room temperature in shaker condition. After 7 days conical flask had a turbid growth with fuzzy fungal growth on top.


Figure 1: enrichment of PSF

### ➢ II. ISOLATION OF PHOSPHATE SOLUBILIZING FUNGI

A loopful from enriched broth was streaked on sterile pikovasky's agar plate. A total 2 plates were streaks to determine the growth of fungi and to evaluate the halo zones produced. After 7 days both the plates had halo zones around the fungal colonies.

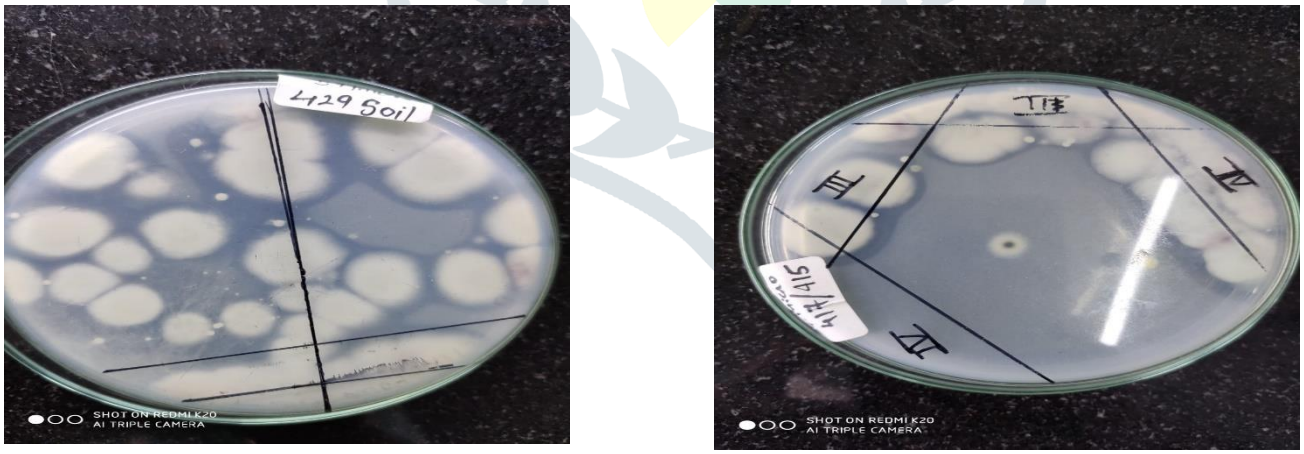


Figure 2: Isolation of phosphate solubilizing fungi

- **III. COLONY CHARACTERISTICS OBSERVED AFTER 7 DAYS OF INCUBATION AT RT.**

| COLONY NO | SHAPE | SIZE | MARGIN | ELEVATION | TEXTURE | COLOUR |
|---|---|---|---|---|---|---|
| 1 | IRREGULAR | 15MM | FILAMENTOUS | RAISED | FUZZY | WHITE |
| 2 | IRREGULAR | 12MM | FILAMENTOUS | RAISED | FUZZY | WHITE |

Table 1: Colony characteristics observed after 7 days.

- **FUNGAL STAINING**

Colonies showing halo zones were selected for fungal staining using lactophenol cotton blue stain.

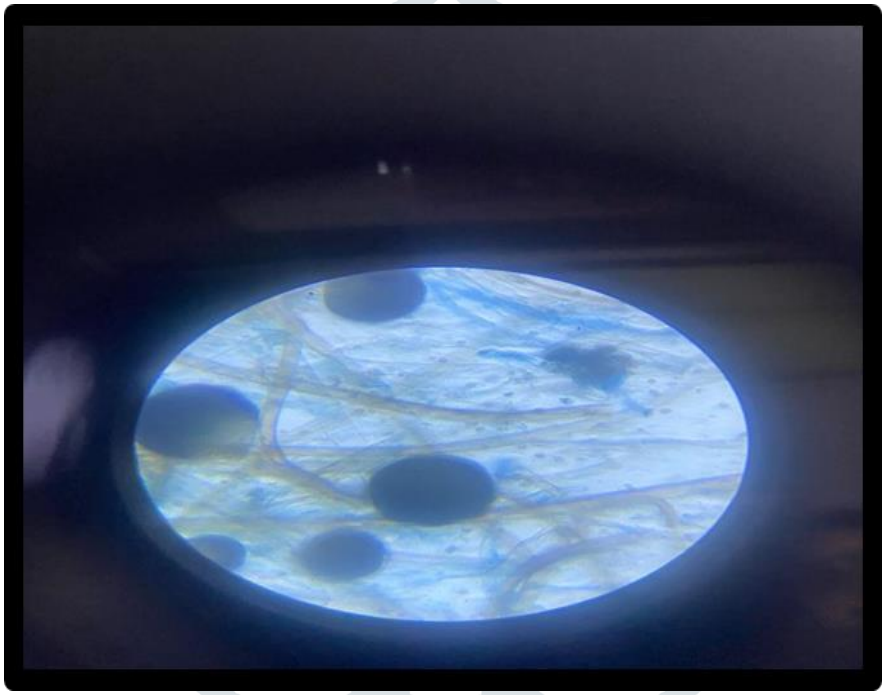

Figure 3: fungal staining showing suggested genus *Mucor*

The selected colonies were subcultured on sabourauds agar slant. To obtain pure culture and stored at 4-degrees Celsius.

- **PREPARATION OF BIOFERTILIZER**

Subcultured colonies were inoculated in pikovasky's broth medium for 7 days at room temperature in shaker condition. After 7 days of incubation fuzzy fungal growth was observed in the conical flask. Isolate 1 and isolate 2 were diluted using distilled water to make different concentration. The concentration of 100%, 75%, 50%, 25%, and Isolate 1 and 2 were mixed and used at concentration of 100% and 50%.

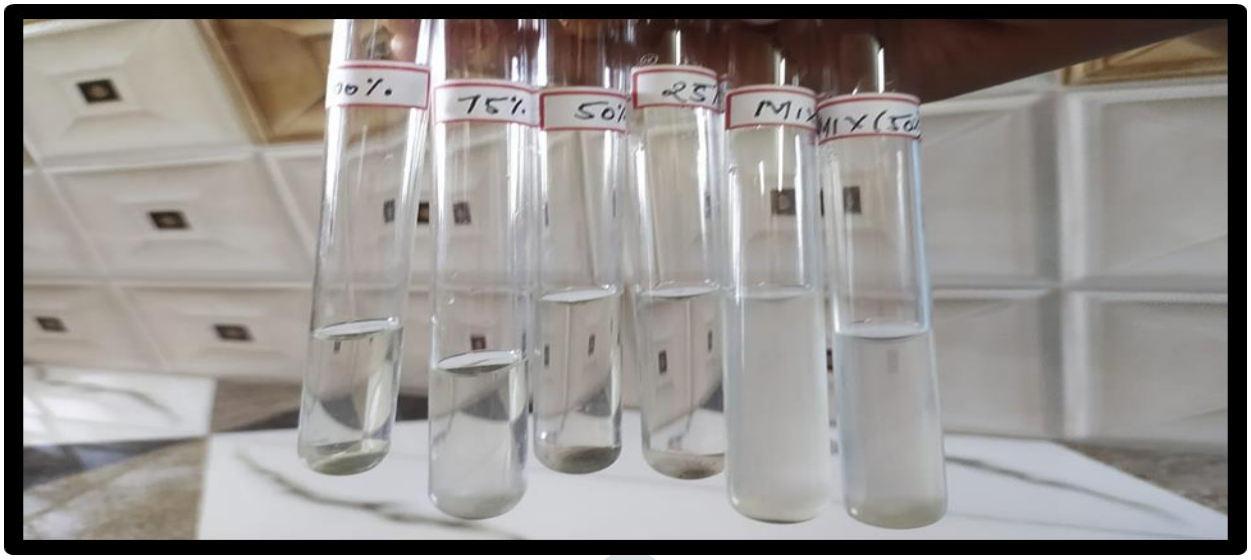


Figure 4: Different concentrations of biofertilizer.

- **PREPARATION OF POT**

Pot mixture was prepared using garden soil and cocopit at 1:1 ratio and label with different concentrations.

- **APPLICATION OF BIOFERTILIZER**

The seeds soaked in different concentration were sowed in soil mixture. The plant was allowed to grow for 7 days in small pots. After 7 days the growth was observed as per concentration and growing length. The pot with 75% concentration of biofertilizer showed the highest growth and the pot with 25% concentration of biofertilizer showed the lowest growth. The rest of all other concentration were with moderate growth with comparison to control which was uninoculated.

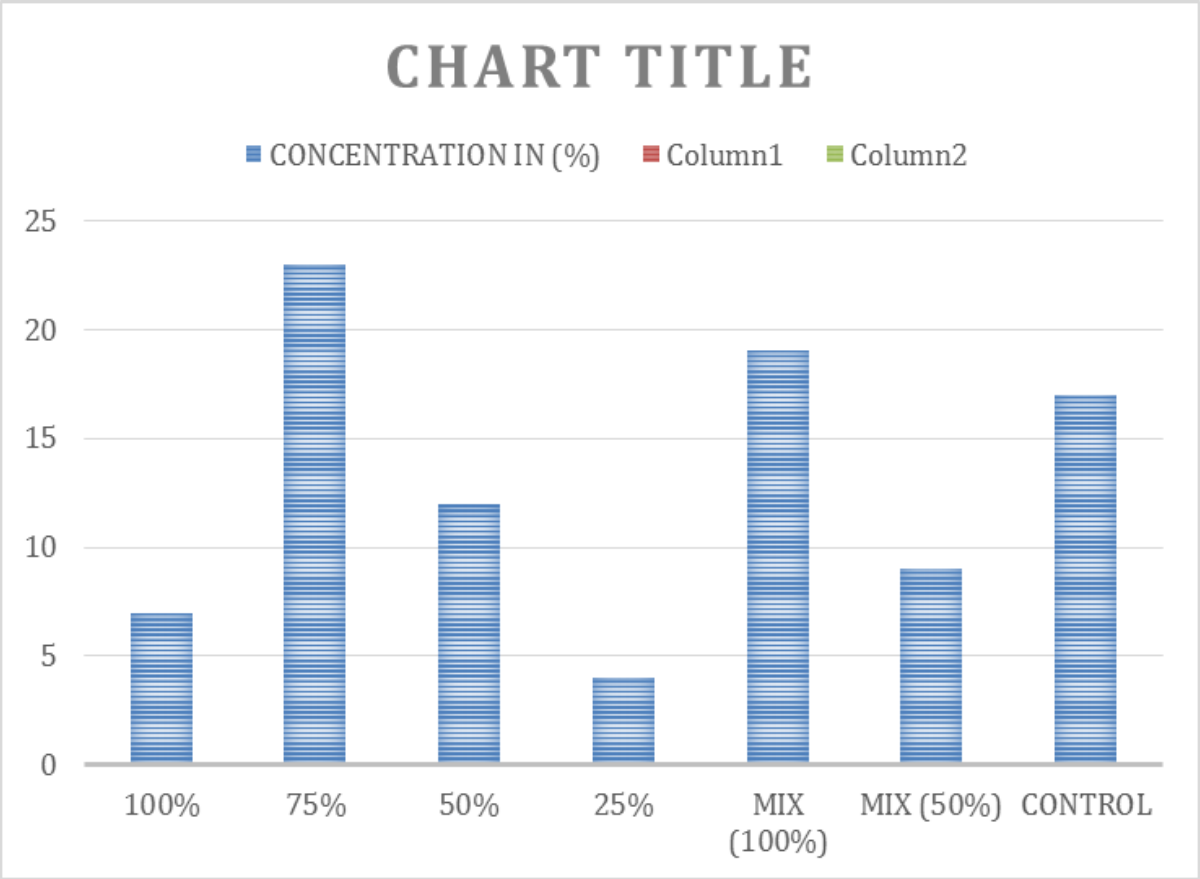


Figure 5: Growth of plant (CM) in different concentrations of biofertilizer.

Figure 6: Plant growth after 7 days with different concentration of biofertilizer.

The study emphasis on biofertilizer for crop production and soil fertility. Agriculture plays a pivotal role in the growth and survival of nations. Therefore, maintaining its quantity and quality is essential for feeding the population and economic exports. Biological fertilization is based on the supply of organic inputs including fertilizers, organic wastes, domestic sewage, animal manure, and microorganisms, such as fungi and bacteria. The bio-fertilizers supply also enhance the productivity per area in a comparatively short time, consume smaller amounts of energy, reduce contamination of soil and water, increase soil fertility, and encourage antagonism and biological control of phytopathogenic organisms. Biofertilizer is a material which contains living microorganisms. When applied to plant surfaces, they promotes plant growth by increasing the supply of

primary nutrients to the host plant. Bio-fertilizers add nutrients through natural processes such as nitrogen fixation, solubilizing phosphorus, and stimulating plant growth along with the synthesis of growth-promoting substances. Bio-fertilizer is technically living; it can be mutually beneficial in association with plant roots. The application of bio-fertilizers can minimize the use of chemical fertilizers, decreasing environmental hazards, enhance soil structure and promote agriculture. Biofertilizers are cheaper and remarkable in affecting the yield of cereal crops. Bio-fertilizers being important components of organic farming play a key role in maintaining long term soil fertility and sustainability by fixing insoluble P in the soil into forms available to plants, thus increasing their effectiveness and availability. In context of both the cost and environmental impact (Sneha et al, 2018).

## V. CONCLUSION AND FUTURE SCOPE

In conclusion, the study was conducted on phosphate solubizing fungi and producing biofertilizer from it. Phosphate solubilizing fungi was isolated from soil and enriched to prepare biofertilizer. Biofertilizer was made using PSF and was tested on moong bean plant. The results showed the higher growth of moong bean plant with biofertilizer than the uninoculated control. The biofertilizer with 75% concentration showed the highest growth rate as compared to other concentration and 25% showed the lowest and poor growth rate Also it is important to understand an exact concentration biofertilizer to be used in farming. Too little and too much can destroy a healthy crop, therefore it is of immense importance to know exact amount on particular crop. The study reveals the growth of plant has been enhanced by the use of biofertilizer.

In the future , enhancement and maintenance of soil fertility through microorganisms will be a very significant concern. Some prospects regarding their future research, commercialization and practical application for sustainable cropping system are critically elucidated. Full evaluation of the potential prospects of biofertilizers for sustainable agriculture and ecosystem will be anticipated globally**.** As the awareness about harmful chemical fertilizers is spreading among the population and willingness to choose an alternate solution is increasing. Biofertilizers will gain enhance recognition.

Campaigns should be conducted in village where the farmers should be provided with biofertilizer samples for free or lesser cost, the consumer of the product such as farmers need to be educated about the biofertilizer and how it’s usage can change the harmful effect caused by chemical fertilizers.

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