EFFICIENCY TEST OF FORMULATED CARRIER BASED BIOFERTILIZER ON *CICER ARIETINUM*

A DISSERTATION SUBMITTED

TO

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FOR THE PARTIAL FULFILMENT OF DEGREE OF

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IN

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BY

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AHMEDABAD - INDIA

This is to certify that the work presented in this Project Report titled ''Efficiency test of formulated carrier based biofertilizer on Cicer arietinum", submitted by SAKSHI DESAI , of Department of Microbiology, Semester IV, has been carried out in the Department of Microbiology, LJ School of Applied Sciences, LJ University, Ahmedabad, during the academic year 2022-23, in partial; fulfilment of the requirement of the degree of Master of Science in Microbiology.

I further certify that this report, or part thereof has not previously been submitted elsewhere for the degree or diploma or any other academic award of this university or any other University.

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I ,**Sakshi Desai**, hereby declare that the Internship/Project Report, titled **"Efficiency test of formulated carrier based bio fertilizer on *Cicer arietinum*."** is submitted by me for the degree of Master of Science in Microbiology, LJ University, Ahmedabad and has not formed the basis of award of any degree, Diploma, Associateship, Fellowship in this University or any other University or Institute of Higher learning. I further declare that the material obtained from other sources, has been duly acknowledged in this report. We shall be fully responsible for any plagiarism or other irregularities noticed in this report.

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INDEX

Abbreviations

N-fixing – Nitrogen fixating

P-solubilising – Phosphate solubilising

PGPR – Plant Growth Promoting Rhizobacteria

EPS – Extracellular polymeric substance

IAA – Indole Acetic Acid

C.F.U – Colony Forming Unit

UV – Ultraviolet

BNF – Biological Nitrogen Fixation

D/W – Distill Water

CHAPTER-1
INTRODUCTION

1.Introduction

The use of fertiliser, which can come from chemical or biological sources, can enhance the condition of the soil. Since the 20th century, chemical fertilisers have been widely used, particularly since they encouraged the Third Agricultural (Green) Revolution.

However, the excessive and widespread use of chemical fertilisers has led to many environmental problems such as air, water, and soil contamination. Chemical fertiliser nitrate concentration can enter aquatic bodies by drainage, leaching, or the and flow. (Savci, 2012)

Eutrophication is brought on by this, which suffocates aquatic life and causes an algal bloom. Additionally, heavy metals like cadmium and chromium are present in chemical fertilisers. Therefore, continued use could cause an accumulation of inorganic substances in the soil, lowering the quality of the soil. The degradation and loss of soil fertility are caused by the ongoing use of chemical fertilisers. This is because it alters the pH of the soil, typically having detrimental effects on soil creatures like worms and soil mites.

The evaporation of ammonia (NH3) from chemical fertilisers, which can oxidise to nitric acid and result in acid rain, also contributes to air pollution. Additionally, nitrogen oxide emissions (NO, N2O, and NO2) have an impact on global warming and climate change.

These negative effects of pesticides raised doubts about how they could improve agricultural productivity while preserving the environment.

With the development of bio fertilizer, the negative effects of chemical fertilisers have been mitigated while providing more benefits. The term "bio fertilizer" refers to fertiliser that includes living microorganisms. It is anticipated that these organisms would affect the soil ecosystem and provide additional nutrients for plants. (DHARMAWAN, 1990)

They contain live, effective formulations of bacteria, algae, and fungi that can fix atmospheric nitrogen, solubilize phosphorus, break down organic matter, or oxidise sulphur. When applied, these formulations will increase the availability of nutrients for the benefit of the plants.

Additionally, they quicken some microbial processes in the soil that increase the amount of nutrients available in an approachable form for plants.

Nobbe and Hiltner created the first type of commercial bio fertilizer, called "Nitragin," in 1895 using nitrogen-fixing rhizobacteria obtained from legumes. Thereafter, *Azotobacter*, blue green algae, and a variety of other microorganisms were discovered. (Ghosh, n.d.)

The soil is a living thing that is home to various organisms. The soil's living characteristics are due to the bacteria that exist there. By adding organic manures to the soil, such as oil cakes, green manure, and cattle manure, the microbial activity is increased. The fixation, solubilisation, and mobilization, among other processes, of nutrients from the atmosphere and soil by soil microbes is crucial for the provision of nutrients to plants. But because chemical fertilizers, pesticides, insecticides, fungicides, and weedicides are consistently used in the soil, all the beneficial microorganisms that are crucial for plant growth, production, and quality have been eliminated. The number and activity of microorganisms in the soil govern its fertility or health, which in turn affects crop output.

The use of laboratory-grown microbial inoculants or microbial cultures has recently drawn attention to sustainable agriculture farming around the world. For crop production, there are two different forms of bio inoculants: bio fertilizers and biocontrol agents. Bio fertilizers are substances that contain microorganisms that are valuable for agriculture and that, through their

biological activity, assist in mobilising plant nutrients. It is an economical and environmentally favourable technique. It is well established that the bio fertilizers promote plant growth and agricultural yield by improving nutrient availability, soil productivity, and sustainability.

Microbial inoculation, also known as bio fertilizer, is a crucial part of organic farming because microbes help to fix atmospheric nitrogen, solubilize and mobilise phosphorous, translocate minor elements like zinc and copper to the plants, produce hormones that promote plant growth, vitamins, and amino acids, and control plant pathogenic fungi. It boosts agricultural output and soil health. Microbial inoculation, also known as bio fertilizer, is a crucial part of organic farming because microbes help to fix atmospheric nitrogen, solubilize and mobilise phosphorous, translocate minor elements like zinc and copper to the plants, produce hormones that promote plant growth, vitamins, and amino acids, and control plant pathogenic fungi. It boosts agricultural output and soil health. (Khan, 2019)

The carrier-based microbial inoculants, often known as "Bio fertilizers," are one of the main components of an integrated plant nutrient system. According to general definitions, bio fertilizers are substances that are applied to seeds or soil that contain live or dormant cells of effective strains of N-fixing, P-solubilizing, or cellulolytic microorganisms.

Several organisms such as *Cyanobacteria, Azolla, Rhizobium,* endophytic diazotrophs and phosphate solubilizing microorganisms are presently being used as bio fertilizers. These microbes are known to improve plant health by secretion of growth promoting substances and by increasing the availability of micronutrients. Phosphate Solubilizing Bacteria especially are slowly emerging as important organisms used to improve soil health as in vitro studies have demonstrated that they bring about dissolution of rock phosphate thereby reducing phosphate deficiency in soil. In the present study phosphate solubilizing bacteria were isolated from soil and their effect on germination. (Med, 2015)

Depending on their forms of activity, bio fertilizers can be grouped into three main categories: N2 fixing bio fertilizers, Phosphate solubilizing/mobilizing bio fertilizers, and plant growth stimulating bio fertilizers.

Bio fertilizers can be either solid or liquid depending on formulation.

CHAPTER-2
REVIEW OF LITERATURE

2.1Summary of the problem

In terms of crop nutrients, nitrogen, phosphorus, and potassium all contribute significantly to increased crop productivity. (Pindi, 2012)

Chemical fertilisers can be effectively replaced by bio fertilizers. However, liquid bio fertilizers are a superior choice because solid bio fertilizers have a short shelf life and a significant danger of contamination.

Additionally, it is necessary to identify and validate the microorganisms that make up bio fertilizer in order to support future study into ways to improve their effectiveness.

2.2 Bio-fertilizer

When given through seed or soil, bio fertilizers, as formulations containing living or latent cells of effective microorganism strains, help crop-plants in absorbing nutrients through their activities in the rhizosphere. (Anubrata & Rajendra, 2014)

Give a brief definition of "bio fertilizer," describing it as "preparations of living cells or effective microorganisms that help in the uptake of nutrients for the growth of plants." (Abdullahi, I N, Isah A D, Chuwang, 2012)

However, Vessey offers a more complete and possibly traditional description. He defines a bio fertilizer as a product that contains living organisms that, when added to soil, seeds, or plant surfaces, colonise the rhizosphere or inside of the plant and aid in growth by increasing the quantity and/or availability of nutrients that pass to the host plant. (Vessey, 2003)

Plant extracts, composted municipal garbage, diverse microbial combinations with unknown components, and chemical fertiliser formulations with organic additives are not considered to be "bio fertilizers" by this definition. These are also known as "fertilisers containing organic matter" or "organic fertilisers."

Bio fertilizers speed up and improve specific microbial soil processes that encourage the availability of nutrients in plant-assimilatable forms. One of the most important components of integrated nutrient management is the use of bio fertilizers, which are affordable, efficient, and sustainable. The soils, water and food sources, animals, and even people have suffered from the excessive and broad usage of petrochemical-based fertilisers. (M. A. Laditi, 2012)

As they are composed of and generated from biological components, bio fertilizers, on the other hand, offer a great substitute for chemical fertilisers. Bacteria, fungus, and/or blue-green algae are just a few of the microorganisms that make up bio fertilizer.

The microorganisms in bio fertilizers add nutrients through the organic processes of nitrogen fixation, solubilisation, and mobilisation of phosphorus through the production of substances that promote growth.

2.3 Classification of bio-fertilizer

Based on their characteristics and uses, bio fertilizers can be divided into four major groups:

1. Bio fertilizers that fix nitrogen / Nitrogen fixing bio fertilizer

2. Bio fertilizer that helps dissolve phosphorus / Phosphorus solubilisation bio fertilizer

3. Bio fertilizer that mobilises phosphate. / Phosphate mobilising bio fertilizer

4. Bio fertilizers that promote plant development / PGPR bio fertilizer

2.3.1 Nitrogen fixing bio-fertilizer

The most crucial nutrient for plant growth is thought to be nitrogen. This is because it is a key ingredient in both amino acids, which serve as the building blocks of proteins, and chlorophyll, the substance that promotes photosynthesis. Although molecular nitrogen makes up around 78% of the atmosphere, fixed nitrogen is a significant nutrient that limits plant growth. (Ohyama, 2010)

Nitrogen from the atmosphere can only be used by plants as ammonia (NH3) through a biological process called nitrogen fixation. The conversion of atmospheric nitrogen into ammonia (NH3) or other molecules that are accessible to living things is a process known as nitrogen fixation.

Nitrogen fixation happens naturally in the air as a result of lightning producing nitrogen oxides (NOX), which can then combine with water to produce nitrous acid or nitric acid, which then seeps into the soil and produces nitrate.

Nitrogen-fixing bacteria can also fix nitrogen biologically, which is responsible for around 90% of nitrogen fixation. They could be free-living, symbiotic, or associative symbiotic microorganisms.

- **Free living nitrogen fixing bacteria:** These include *Azotobacter*, *Clostridium*, and cyanobacteria (blue-green algae). The enzyme nitrogenase is responsible for reducing atmospheric nitrogen to ammonia, a process known as nitrogen fixation. Nitrogenase consumes a lot of energy. The nutrients required to supply this energy are acquired by bacteria that are free-living. They are particularly crucial in fixing nitrogen for crops like maize and wheat that do not benefit from symbiotic bacteria, although existing at relatively low quantities. (Burk et al., 1934)
- **Symbiotic nitrogen fixing bacteria:** These bacteria, which are also referred to as mutualistic, establish a beneficial interaction with the plants' roots. Exudates, which are sugars that serve as both a carbon source and an energy source for the bacteria, are nutrients that the plant gives to them. In response, the bacteria infiltrate the root hair, where they multiply and promote the creation of root nodules. There, they convert free nitrogen to ammonia, which the host plant uses for growth. Leguminous species (like beans and peas) frequently have this association, which ensures the best possible growth for them.
- **Associative symbiotic nitrogen fixing bacteria:** In the host plant, this particular bacterial group does not create symbiotic structures. However, they penetrate the host's brain and vascular tissues and promote the formation of new lateral root hairs. This leads to an increase in mineral absorption, which is caused by the formation of phytochrome. Grass is where this particular class of bacteria is more common.

2.3.2 Phosphate solubilising bio-fertilizer

Phosphorus is the second most important plant nutrient that limits crop yield. In addition to photosynthesis and genetic transfer, it is essential for almost every plant process that involves the transfer of energy. Phosphorus deficiency inhibits plant growth and size.

Although it occurs primarily in insoluble forms and is abundant in soils in both organic and inorganic forms, its availability is limited. Only around 0.1% of the total phosphorus in soil is soluble and accessible to plants. (Sharma et al., 2013)

The diverse strains of bacteria and fungi that make up phosphate-solubilizing bio fertilizers have been proven to solubilize phosphate. Although these organisms are everywhere, their density and capacity for mineral phosphate solubilisation varies from one soil or production system to another.

2.3.3 Phosphate mobilising bio-fertilizer

Phosphorus is an inert nutrient. The greatest barrier to phosphorus uptake in the majority of plants is the movement of phosphorus to the root, not phosphorus uptake by the root from the soil.

Orthophosphates (Pi), a derivative of phosphorus, are delivered to the roots through diffusion rather than to mass flow, and their rate of soil diffusion is modest. The symbiotic link between plant roots and mycorrhizae helps to overcome this obstacle. This connection is based on the mutually beneficial transfer of nutrients between the roots of vascular plants and soil fungus. The plant provides sugars from photosynthesis to the fungi, and the hyphae network increases the plant's ability to absorb water and nutrients, particularly phosphorus. (S. E. Smith et al., 2003)

2.3.4 Plant Growth Promoting Rhizobacteria

They are also known as microbial pesticides, such as *Pseudomonas fluorescens* and *Bacillus spp. Serratia spp.* and *Ochrobactrum spp.* are capable of promoting plant growth, while *P. fluorescens* applied to black pepper increases nutrient intake, which increases plant biomass.

It was discovered that rhizobacteria could boost plant development both directly and indirectly by altering the microbial balance in the rhizosphere in favour of beneficial microorganisms and by generating growth hormones and enhancing nutrient intake. (Bevivino et al., 1998)

2.4 Types of bio-fertilizers

Bio fertilizers are currently available in both solid and liquid forms.

2.4.1 Solid carrier bio-fertilizer

This includes a carrier substance that serves as a vehicle for the microorganisms to be used as bio fertilizer for a future seed or soil inoculation. (Satinder, 2012)

These substances may contribute to the preservation of the microorganisms' viability (shelf life) before they are released into the environment as well as to the creation of an ideal like soil, paddy straw compost, peat, vermiculite, lignite powder, clay, talc, rice bran, seed, rock phosphate pellets, wheat bran, or a combination of these substances.

A carrier material or combination of carrier materials is often chosen based on the viability of the microorganisms established with it to improve the shelf-life of bio fertilizer formulations. The carrier material used to make seed inoculant is fine powder.

According to "Handbook for Rhizobia", a good carrier medium for seed inoculation has the following qualities:

(i) The inoculant bacteria strain is not hazardous to it.

(ii) A strong ability to absorb moisture.

(iii) Simple to process and devoid of substances that cause lumps.

(iv) It is simple to sterilise using an autoclave or gamma radiation.

(v) Ample supply is offered.

(vi) Affordable.

(vii) Strong seed adherence.

(viii) Good pH buffering capacity.

2.4.2 Liquid bio-fertilizer

A group of microorganisms that have been given an appropriate liquid medium to maintain their viability for a set amount of time is known as a liquid bio fertilizer. This helps to increase the biological activity of the target site. (Pindi, 2012)

Liquid bio-fertilizers are defined as special liquid formulations that contain not only the required microorganisms and their nutrients but also unique cell protectants or compounds that encourage the creation of inactive spores or cysts for a longer shelf life and tolerance to harsh conditions.

In order to increase crop productivity, liquid bio fertilizers contain living species of microorganisms that can mobilise nutritionally significant elements through biological processes like nitrogen fixation, phosphate solubilisation or mobilisation, excretion of plant growth-promoting substances, or cellulose or lignin degradation from non-usable to usable forms.

A problem with solid-carrier bio fertilizer is that the microorganisms have a six-month shelf life and an initial population density of 108 c.f.u. per millilitre, intolerance to UV radiation, and intolerance to temperatures above 30 °C. However, liquid bio fertilizers are able to solve these problems.

Liquid bio fertilizers contain microorganisms with an average shelf life of two years, a population count of up to 109 c.f.u./ml, and the ability to withstand high temperatures (up to 55°C) and UV radiation.

2.5 Why bio-fertilizers should be used instead of chemical fertilizers?

- Chemical fertilizers, have decreased soil health by preventing soil microflora and microfauna, which are largely responsible for maintaining soil fertility.
- The extensive use of chemical fertilisers harms the environment and has negative effects on living things.
- These dangerous chemicals cannot be absorbed by plants, they begin to build up in ground water, and some of these chemicals are also to blame for the eutrophication of water bodies.
- These substances have negative effects on soil in terms of decreased soil fertility, increased salinity, and uneven distribution of nutrients. (Mahanty et al., 2016)
- Whereas, the excessive use of chemical fertilisers in agriculture is expensive and has negative impacts on the physico-chemical characteristics of soils.
- As a result, various organic fertilisers that work as natural stimulators for plant growth and development have been introduced.
- The knowledge of such natural stimulators or microbial inoculums has a lengthy history that began with the culture of small-scale compost production and is passed down from farmer to farmer.
- Bio-fertilizers, also known as "microbial inoculants," are preparations that contain living or latent cells of effective strains of nitrogen fixing, phosphate solubilizing, etc.
- Due to their affordability, environmental friendliness, and availability as a renewable source of plant nutrients, bio-fertilizers are increasingly replacing chemical fertilisers.
- Bio-fertilizers are substances that contain one or more species of microorganisms that are capable of mobilising nutritionally significant elements through biological processes like nitrogen fixation, phosphate solubilisation, excretion of plant growth-promoting substances or cellulose, and biodegradation in soil, compost, and other environments from non-usable to usable form.
- In the current environment of rising fertilizer costs and their harmful impacts on soil health, the use of bio-fertilizers in agriculture assumes considerable significance.
- Thus, there are basically two causes for the use of bio-fertilizer. First, because greater use of fertilisers results in higher agricultural yields; second, because greater use of chemical fertilisers damages the texture of the soil and causes other environmental issues. So, one should use bio-fertilizer**.** (Kumawat, 2018)

2.5.1 Advantages of using bio fertilizers

- They are both affordable and environmentally friendly.
- They are used to enrich the soil; over a time period it will improve the quality of soil.
- These fertilisers capture the nitrogen from the air and deliver it right to the plants.
- By releasing and solubilizing phosphorus they increase the soil's phosphorous concentration.

- Due to the release of growth-stimulating hormones by biofertilizers it increases root proliferation.
- For the benefit of the plants, microorganisms transform complicated nutrients into simple nutrients.
- The microorganisms in biofertilizer help to ensure that the host plants receive an enough amount of supply of nutrients and that their growth is properly regulated.
- To certain point, biofertilizers can shield plants against diseases that are transmitted through the soil.
- They increase the yield of crop by 10-25%. (Kumawat, 2018)

2.5.2 Restrictions in using bio fertilizer

- **Lack of an appropriate strain:** Biofertilizer output is reduced due to a lack of an appropriate strain.
- **Lack of a suitable carrier:** Biofertilizer's shelf life is not maintained in the absence of a suitable carrier.
- **Lack of knowledge among farmers:** Indian farmers are unaware of the benefits of using biofertilizers to raise crops. (Ritika & Utpal, 2014)

2.5.3 Limitations of biofertilizer

- Chemical fertilisers and biofertilizers do not mix.
- Fungicide and paper ash are not sprayed with biofertilizers at the same time. (Ritika & Utpal, 2014)

2.6 Crop

Pulses are a significant dietary protein source and have the special ability to conserve and improve the physical qualities of the soil due to their deep root systems and leaf fall as well as preserve and restore soil fertility through biological nitrogen fixation.

Due to their capability to grow on minor areas and supply a protein-rich diet to the majority of the country's vegetarian population, pulses serve a crucial function and occupy a special position in Indian agriculture. Pulses and grains together boost the biological value of the protein eaten. Pulses are essential to sustainable agriculture because they enhance the soil and function as a small-scale nitrogen factory in soil. Most commonly, they are eaten as dal and processed whole seeds. These ingredients are used to make a wide range of snacks, desserts, and sauces that are excellent blood purifiers and remedies for stomach problems.

2.6.1 Chickpea

The chickpea is the second-largest pulse crop in the world and is grown in at least 33 nations in South Asia, West Asia, North Africa, East Africa, southern Europe, North and South America, and Australia. It makes up 14% (7.9 million tonnes) of the world's total production of pulses and occupies 15% (10.2 million hectares) of the total area. Chickpeas offer high-

quality protein, especially for vegans and people who cannot afford meat, in the cereal-heavy diets of South, West, and East Asia as well as North Africa**.** (K.B. Singh, 1997)

The chickpea, or Cicer arietinum, is an edible legume belonging to the Fabaceae family, subfamily Faboideae. Rice farming can be replaced with chickpeas in regions with inadequate irrigation to increase soil fertility and control weeds. The ancient world's first domesticated grain legume was Cicer arietinum L. Most likely, a region of today's south-eastern Turkey and surrounding portions of Syria is where the chickpea originated**.** (Bidyarani et al., 2016)

Chickpeas are a good source of minerals such calcium, phosphorus, iron, and vitamins as well as 18–22% protein, 52%–70% carbohydrate, and 4–10% fat. It is also important for sustainable agriculture because it enhances the soil's physical, chemical, and biological qualities. As a result of its deep roots and improved soil aeration, leaf fall increases the amount of organic matter in the soil. As a result, chickpea is essential for enhancing soil health.

Fig 1- Growth Stages of Chickpea Plant.

2.7 Plant Growth Promoting Rhizobacteria (PGPR)

- PGPR are a diverse group of bacteria that can be found in the rhizosphere, at root surfaces, and in connection with roots. They can directly and/or indirectly enhance the extent or quality of plant growth.
- The connection between plants and microbes, particularly plant growth-promoting rhizobacteria (PGPR), is greatly promoted by the soil in the rhizosphere.
- PGPs are known to quickly colonise the rhizosphere and to reduce soil-borne pathogens at the root surface.
- They are beneficial to plant by increasing their growth through different direct and indirect mechanisms.(Dasgupta, Ghati, Sarkar, et al., 2015)
- There are various direct and indirect mechanisms of plant growth promotion performed by different PGPR strains.

2.7.1 Direct mechanisms

Nitrogen fixation

- All life depends on nitrogen, which is also the most important nutrient for plant development and productivity.
- There is 78% more nitrogen in the atmosphere than there is in the soil, so plants cannot get it.
- Unfortunately, no plant species has the ability to convert atmospheric nitrogen into ammonia and use it for growth.

- Thus, biological nitrogen fixation (BNF), which converts atmospheric nitrogen to ammonia by nitrogen-fixing microorganisms via a complicated enzyme system known as nitrogenase, transforms atmospheric nitrogen into plant-utilizable forms.
- PGPR strains have ability to fix atmospheric nitrogen by symbiotic and non-symbiotic form.
- Symbiotic nitrogen fixation occurs when a microorganism and a plant have a mutualistic interaction. The microorganism first enters the root, then creates nodules where nitrogen fixing takes place. Example: - *Rhizobia,* etc.
- On the other hand, diazotrophs that are free to live do non-symbiotic nitrogen fixation, which can promote the growth of plants other than legumes. Example:- *Azotobacter, Azospirillum,* etc. (Gupta et al., 2015)

Phosphate solubilisation

- In terms of plant nutrition, phosphorus is second only to nitrogen (N) in importance. It is crucial to nearly all of the key metabolic functions of plants, including photosynthesis, energy transfer, signalling, macromolecular biosynthesis, and respiration.
- As phosphate is present in insoluble form so, 99% of phosphate cannot be absorbed by plants.
- Only two soluble forms of phosphate: - monobasic (H2 PO4) and diabasic (HPO4 2-) ions—are absorbed by plants.
- There are 3 different mechanisms for phosphate solubilisation through PGPR bacteria.
  (1) Release of chemicals that complicate or dissolve minerals.
  (2) Release of extracellular enzymes.
  (3) The discharge of phosphate during substrate breakdown.
- Example:- *Arthrobacter, Bacillus,* etc. (Gupta et al., 2015)

Potassium solubilisation

- The third most important macronutrient for the growth of plants is potassium (K). Most of the time, soil has relatively little soluble potassium, and more than 90% of the soil's potassium is present in the form of silicate minerals and insoluble rocks.
- Through the synthesis and secretion of organic acids, plant growth-promoting rhizobacteria can solubilize potassium rock.
- Example:- *Acidothiobacillus , Bacillus, Pseudomonas,* etc. (Gupta et al., 2015)

Indole acetic acid

- The most prevalent naturally occurring auxin found in plants is indole acetic acid (IAA), which has a beneficial impact on root growth.
- Indole acetic acid influences plant cell division, extension, and differentiation; promotes seed and tuber germination; quickens the development of xylem and roots; regulates the processes of vegetative growth; starts the formation of lateral and adventitious roots; mediates responses to light, gravity, and

florescence; and influences photosynthesis, pigment formation, the biosynthesis of different metabolites, and stress tolerance.

- The major precursor molecule for the production of IAA in bacteria has been identified as the amino acid tryptophan, which is frequently present in root exudates.
- Example:- *Pseudomonas, Rhizobium,* etc. (Gupta et al., 2015)

2.7.2 Indirect mechanisms

Antibiosis

- Synthesis of antibiotics is most effective biocontrol method of PGPR bacterial strains against phytopathogens.
- One issue with relying too heavily on rhizobacteria that promote plant development and produce antibiotics as biocontrol agents is that some phytopathogens may become resistant to particular antibiotics as a result of greater use of these strains. Some researchers have used biocontrol strains that synthesise one or more antibiotics to stop this from developing. (Gupta et al., 2015)

Siderophore

- Living things require iron as a cofactor for growth. At neutral and alkaline pH, soils' solubilized ferric ion availability to soil microorganisms is constrained.
- Fe3+ is seized in the vicinity of the root to stop the growth of harmful bacteria.
- Several bacterial siderophores can be used as iron sources by many plants, although the overall concentrations are probably too low to significantly affect plant iron intake.
- Example:- *Serretia, Pseudomonas, Rhizobium,* etc. (Gupta et al., 2015)

Exopolysaccharide production

- Making of exopolysaccharide is important for production of biofilm.
- Protection from stress, shielding from desiccation and many other are the functions of EPS producing microbes.

  Exopolysaccharide produced by PGPR strains are helpful in increasing plant growth because it is an active signal molecule. (Gupta et al., 2015)

2.8 Seed germination

- The activation of seed enzymes during seed germination affects how high molecular weight materials in the seed degrade into low molecular weight materials.(Safonova et al., 2018)
- While in some seeds growth begins as soon as water is absorbed, in others germination does not occur until certain conditions are met.
- The three distinct stages of seed germination are (a) water intake, (b) cell lengthening, and (c) cell proliferation.

- There are two types of seed germination.(1) Hypogeal Germination, (2) Epigeal Germination.
- Epigeal germination a form of germination in which the hypocotyl lengthens quickly and arches upward, drawing the cotyledons up out of the ground.
- Hypogeal germination puts the growing seedling below the surface of the ground, which lessens the impact of freezing and other desiccating climatic conditions**.**(Shahid et al., 2013)

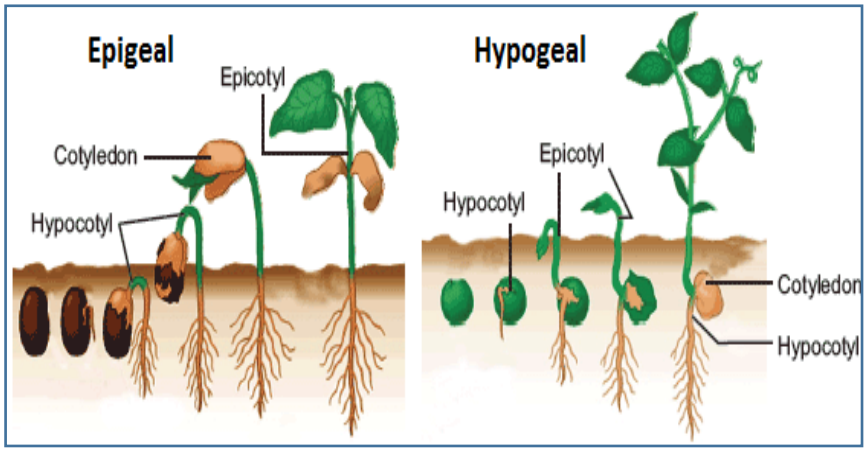


Fig 2- Epigeal and Hypogeal Germination

2.9 Pot study

- In addition to field observations, pot experiments allow for the controlled study of plants without the effects of different environmental conditions.
- Tree experiments in pots posess more issues than herbaceous plants because of the bigger dimensions of tree species.
- Pot experiments have the benefit of allowing direct measurements under controlled circumstances without the effect of distracting biotic and abiotic elements.(Kawaletz et al., 2014)

CHAPTER-3
OBJECTIVES

3. Objectives

General objective:

- To study the effect of formulated carrier based biofertilizer on Chickpea.

Specific objective:

- Isolate the bacteria from previously prepared biofertilizer.
- To study the PGPR characteristics of our isolated bacteria.
- To study the efficiency of formulated carrier based biofertilizer on Chickpea.
- To analyse different plant growth parameters.

CHAPTER-4
DESIGN OF EXPERIMENT

4.1 Plan of work

Activate the bio fertilizer culture that was created during the agricultural module.

↓

From activated culture, isolate the required bacterial culture or colony.

↓

To determine its PGPR properties, do PGPR tests.

↓

Pick the crop you want to investigate.

↓

For a rough estimate of how long it will take a seed to grow in a pot, perform seed germination.

↓

To see how biofertilizer affects our crop, conduct a greenhouse study.

↓

Study the results of different plant growth parameters.

↓

Observe the results.

↓

Conclude your results.

4.1.1 Sampling

Collection of soil sample

We have collected garden soil of L.J. Campus as our sample. It was collected in an alcohol wiped/sterilized zip lock bag.

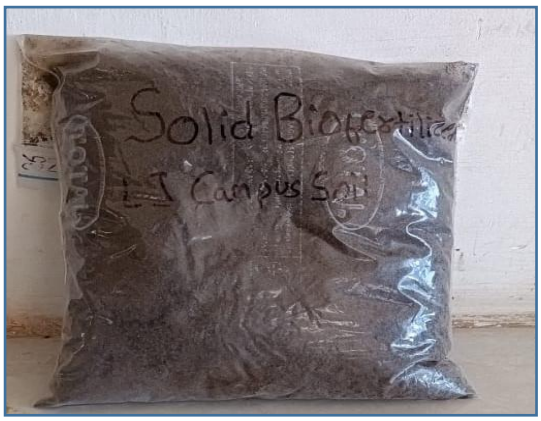


Fig-3 Soil sample collected from L.J campus

4.2 Materials

> For preparation of phosphate bio fertilizer -Soil sample -Pikovskaya plate -Pikovskaya slant -Nutrient broth -Sugarcane bagasse -Vortex -Rotatory shaker -Incubator	**> For activation of phosphate solubilizing bacteria** -Pikovskaya broth -Phosphate Solubilizing Bacteria -Rotatory Shaker
> For isolation of phosphate solubilizing bacteria -Pikovskaya plate -Bromophenol blue -Culture -Dilution tubes -Incubator -Spreader	**> For microscopy of isolated bacteria** -Bacterial culture -Gram's iodine -Crystal violet -Safranin -Alcohol (decolorizing agent) -Oil -Microscope
> For phosphate solubilisation -Pikovskaya plate -Bromophenol blue -Incubator -Dilution tube -Spreader	**> For ammonia production** -2% peptone water -Red litmus paper -Incubator -Nessler's reagent -Spectrophotometer
> For indole acetic acid production -Luria Bertani broth -Tryptophan -Salkowaski reagent -Incubator -Centrifuge tubes -Centrifuge -Spectrophotometer	**> For hydrogen cyanide** -Nutrient agar slant -Glycine -Picric acid strips -Incubator
> For EPS production -Nutrient broth -2% sucrose -Incubator -Whatmann filter paper -Hot air oven	**> For nitrogen fixation** -Ashby's Mannitol agar plates -Incubator -Spreader
> For seed Germination -Nutrient broth -Rotatory shaker -Distill water -Moist chamber -Seeds	**> For pot study** -Nutrient broth -Rotatory shaker -Neuberg's chamber -Seeds -Pot
> For chlorophyll estimation -Leaves -Centrifuge -Mortar pestle -50 ml Volumetric flask -80% acetone -Spectrophotometer -Centrifuge tube	**> For length and fresh and dry weight of root and shoot, no of leaves, nodes and internodes** -Uprooted plant -Hot air oven -Weigh balance

4.3 Methodologies

4.3.1Preparation of phosphate bio-fertilizer

Step-1: Inoculate 1 gm of soil sample to autoclaved 20 ml Pikovskaya broth.

Step-2: Incubate at 37 C in rotary shaker for 24 hours.

Step-3: Transfer bacterial growth to Pikovskaya plate.

Step-4: Incubate at 37 C for 24 to 48 hours.

Step-5: Transfer different colony to Pikovskaya slant to get pure culture.

Step-6: After that we performed PGPR Characteristic test for identifying properties of our pure culture organisms.

Step-7: A single colony from each culture was inoculated in 10 ml nutrient broth.

Step-8: Incubate over night with continuous shaking (120 RPM) at optimum temperature.

Step-9: Liquid cultures of each bacterium transferred to 300 ml nutrient broth to scale up and constitute active consortium.

Step-10: Then centrifuge at 10,000 RPM for 10 minutes.

Step-11: Sterilization of sugar cane bagasse in hot air oven at 100 c for 15 min.

Step-12: Bacterial pallets were incorporated with 20 gm sterilized sugar cane bagasse under aseptic conditions.

Step-13: The mixture was vortexed for 45 minutes in support of homogeneous mixing of sugar cane and bacterial cells and dried at room temperature (28 C).

Step-14: After complete drying sugar cane bagasse based formulations were packed in clean air tight sterilized packets and sealed separately.

Fig – 4

Fig – 5

Fig 4 & 5 – Bio fertilizer

4.4 Activation and isolation of microorganism

4.4.1 Activation of phosphate solubilizing bacteria

Step-1: Make 50 ml of Pikovskaya broth in a 100 ml conical flask.

Step-2: Autoclave the media and let it cool down at room temperature.

Step-3: Inoculate 1 gm of Phosphate solubilizing bio fertilizer.

Step-4: Incubate the flask on shaker for 24-48 hrs at 37 C on rotatory shaker.

Step-5: Observe the growth.

Fig-6 Activating the culture from bio fertilizer

4.4.2 Isolation of phosphate solubilizing bacteria

Step-1: Make Pikovskaya agar plates containing pinch of bromophenol blue dye.

Step-2: Autoclave the media and let it cool down at room temperature.

Step-3: Inoculate 0.1ml of culture on Pikovskaya plate by serial dilution method and spread plate method. (Respective cultures on respective plates).

Step-4: Incubate the plates at 37 C for 24-48 hrs in inverted position.

Step-5: Observe the growth of phosphate solubilizing bacteria and zone of phosphate solubilisation.

4.5 Microscopy of isolated bacteria

Step-1: Make the smear of bacterial culture and heat fix it.

Step-2: Crystal violet staining solution is flooded onto an air-dried, heat-fixed smear of cells for 1 minute.

Step-3: Slide should be washed for two seconds in a mild flow of water.

Step-4: Flood slide with Gram's iodine and allow it to stain for 1 minute.

Step-5: Slide should be washed for two seconds in a mild flow of water.

Step-6: Flood the slide with decolorizing agent for 15 seconds.

Step-7: Flood the slide with safranin and counterstain. Wait between 30 and 60 seconds.

Step-8: Slide should be washed in a gentle flow of tap water until there is no longer any colour. After washing, blot slide dry with absorbent paper.

Step-9: Under immersion in oil, evaluate the staining process's results. Gram-positive bacteria stain blue/purple, while gram-negative bacteria stain pink/red. (A. Smith & Hussey, 2020)

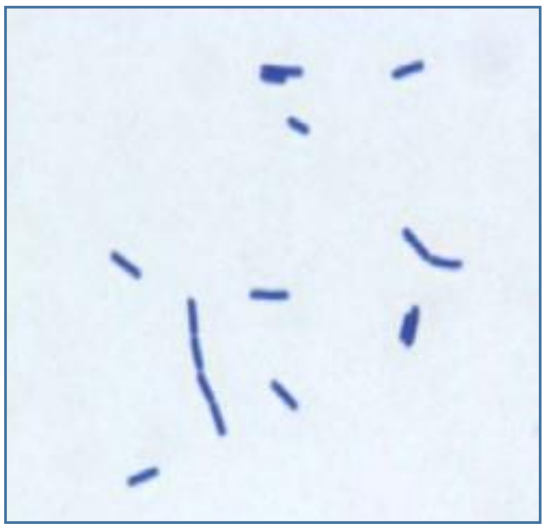


Fig-7 Gram positive bacteria

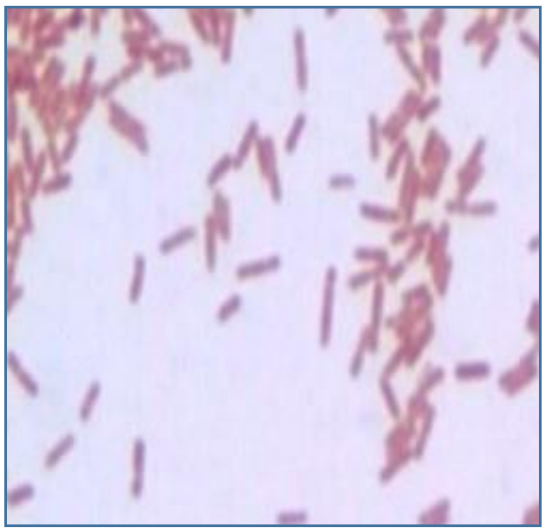


Fig-8 Gram negative bacteria

4.6 PGPR characterisation

4.6.1 Phosphate solubilisation

Step-1: Prepare Pikovskaya agar plates containing pinch of bromophenol blue dye.

Step-2: Prepare dilutions of our culture.

Step-3: Inoculate 0.1 ml of sample by spread plate method.

Step-4: Incubate the plates at 37C for 24-48 hrs in inverted position.

Step-5: Observe the yellow coloured zone of around the colony.

Step-6: Measure the diameter of colony and the diameter of solubilisation zone.

Step-7: Calculate its solubilisation efficiency and solubilisation index.

Formula to calculate Solubilisation Efficiency(SE) and Solubilisation Index(SI) are as follow:

$Solubilization\ Efficiency = \frac{Diameter\ of\ zone}{Diameter\ of\ colony}$ X 100 (Med, 2015)

$Solubilization\ Index = \frac{Diameter\ of\ colony + Diameter\ of\ zone}{Diameter\ of\ colony}$ (Singh et al., 2014)

4.6.2 Ammonia production

Qualitative estimation

Step-1: Prepare 2% Peptone Water tubes (2gm/100ml).

Step-2: Inoculate cultures to peptone water.

Step-3: Put a strip of red litmus paper in a test tube containing inoculated peptone water.

Step-4: Incubate the tubes at 30C with continuous shaking at 140-150 rpm for 5 days.

Step-5: Add 1ml of Nessler's reagent to all tubes.

Step-6: Positive result – Appearance of yellow to dark brown colour.

Colour change of red litmus paper to blue.

Quantitative estimation

Step-1: Prepare 2% Peptone Water tubes (2gm/100ml).

Step-2: Inoculate cultures to peptone water.

Step-3: Incubate the tubes at 30C with continuous shaking at 140-150 rpm for 5 days.

Step-4: Add 1ml of Nessler's reagent to all tubes.

Step-5: Take the O.D. of the mixture at 540nm using a spectrophotometer. (Use uninoculated peptone water + 1ml Nessler's reagent as control) (Kamaruzzaman et al., 2020)

4.6.3 Hydrogen cyanide production

Step-1: Prepare Nutrient Agar slant containing glycine (4.4g/l).

Step-2: Prepare picric acid strips using whatmann filter paper no.1.

Step-3: Inoculate the cultures on slant by streaking.

Step-4: Incubate the slants at 30C for 48 hrs.

Step-5: Positive result – Colour change of picric acid strip from yellow to reddish brown colour. (Rana et al., 2011)

4.6.4 Indole acetic acid production

Qualitative estimation

Step-1: Prepare Luria Bertani broth containing tryptophan and Salkowaski reagent.

Step-2: Inoculate the broth with culture.

Step-3: Incubate the inoculated broth at 28C for 72 hrs.

Step-4: After 72 hrs, Centrifuge the broth at 5000rpm for 20 mins / 10,000rpm for 10 mins.

Step-5: Collect 2ml supernatant in a test tube and add 2m salkowaski reagent to the tube.

Step-6: Incubate the tubes in dark condition for 30 mins.

Step-7: Positive Result – Appearance of pink colour.

Qualitative estimation

Step-1: Prepare Luria Bertani broth containing tryptophan and Salkowaski` reagent.

Step-2: Inoculate the broth with culture.

Step-3: Incubate the inoculated broth at 28C for 72 hrs.

Step-4: After 72 hrs, Centrifuge the broth at 5000rpm for 20 mins / 10,000rpm for 10 mins.

Step-5: Collect 2ml supernatant in a test tube and add 2m salkowaski reagent to the tube.

Step-6: Incubate the tubes in dark condition for 30 mins.

Step-7: Observe appearance of pink colour.

Step-8: Take the O.D at 530nm. (M. et al., 2017)

4.6.5 EPS

Step-1: Prepare Nutrient Broth containing 2% sucrose (2gm/100ml).

Step-2: Inoculate the broth with culture.

Step-3: Incubate the broths at 37C for 48hrs.

Step-5: Take a whatmann filter paper and measure its initial weight (W_1).

Step-6: Filter the broth using previously measured filter paper.

Step-7: Dry the filter paper in hot air oven for approximately 30-45 min at 50C.

Step-8: Measure the dry weight of filter paper (W_2).

Step-9: Calculate the amount of EPS produced.

$$EPS\ produced = W_2 - W_1$$

4.6.6 Nitrogen fixation

Step-1: Prepare Ashby's Mannitol Agar plates.

Step-2: Prepare suspension of our culture.

Step-3: Inoculate 0.1 ml of sample from suspension by spread plate method.

Step-4: Incubate the plates at 37C for 72-96 hrs in inverted position.

Step-5: Positive Result: Appearance of dew drop like colony.

4.7 Seed germination

Step-1: Prepare Nutrient broth.

Step-2: Autoclave broth, petri plates and blotting paper.

Step-3: Inoculate broth with the isolated culture.

Step-4: Incubate the broth for 72-96 hrs at 37C on rotatory shaker.

Step-5: After incubation, soak the seeds in the broth and in D/W for control for 24hrs/overnight.

Step-6: Prepare a moist chamber by placing a moist blotting paper in petri plates.

Step-7: Remove the seeds from the broth and place it in the moist chamber.

Step-8: Observe the growth of radicle and plumule for 15 days.

Step-9: Calculate the germination percentage.

$Germination\ percentage = \frac{Germinated\ Seeds}{Total\ seeds}$ X 100 (Kaur et al., 2018)

4.8 Pot study

Step-1: Prepare Nutrient broth and autoclave it.

Step-2: Inoculate the broth with the isolated cultures.

Step-3: Incubate the broth on 37C on rotatory shaker for 72-96 hrs.

Step-4: Measure the count of cell by using neuberg's chamber.

Step-5: After having appropriate cell count, soak the seeds in broth for 24hrs/overnight.

Step-6: Fill the pot with approximately 2kg soil.

Step-7: Sow the seeds at equidistance and about 1inch deep.

Step-8: Regularly water the plants and observe the growth.

Step-9: Uproot the plants after 20 days and analyse plant growth parameters.

4.9 Plant growth parameters

Plants form each pot were randomly harvested to analyse its growth.

4.9.1 Chlorophyll estimation

Step-1: Add 1 gm of finely cut leaves to a mortar pestle.

Step-2: Add 20 ml of 80% acetone to a mortar pestle and make a fine pulp of leaves.

Step-3: Fill the pulp in 10 ml centrifuge tube.

Step-4: Centrifuge the pulp at 5000rpm for 5 min.

Step-5: Collect the supernatant in a 50ml volumetric flask.

Step-6: Make final volume of 50ml by filling it with 80% acetone up to the mark.

Step-7: Take the absorbance of extract at 663 and 645 nm.

Step-8: Calculate the amount of chlorophyll a, chlorophyll b, and total chlorophyll. (Kizhedath & Suneetha, 2011)

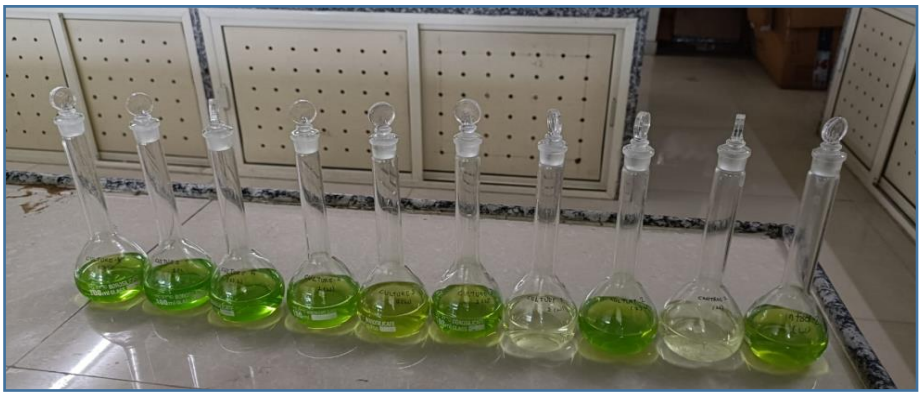


Fig-9 Chlorophyll Estimation

4.9.2 Length of root and shoot

Step-1: After uprooting the plants, separate the root part and shoot part of the plant.

Step-2: Measure the length of root and shoot. (Karnwal & Kumar, 2012)

4.9.3 Fresh and dry weight of root and shoot

Step-1: After uprooting the plants, separate the root part and shoot part of the plant.

Step-2: Immediately, after harvesting take the fresh weight of root and shoot.

Step-3: Then put the root and shoot in the hot air oven at 50-60 C for 30 mins and allow it to dry.

Step-4: Take the dry weight of root and shoot. (Karnwal & Kumar, 2012)

4.9.4 Number of leaves, nodes and internodes

Step-1: After uprooting the plants, separate the root part and shoot part of the plant.

Step-2: Count the number of nodes.

Step-3: Count the number of internodes.

Step-4: Pluck the leaves and count its number. (Karnwal & Kumar, 2012)

CHAPTER-5
RESULTS

5.1 Isolation of phosphate solubilizing bacteria

After obtaining phosphate solubilizing bacterial growth on Pikovskaya plate through spread plate method. The cultures were streaked on Nutrient Agar plates by four flame method to obtain its isolated and pure growth. Total 8 isolates were obtained out of them 4 isolates were selected for further studies. They were named as P-2, P-3, P-4 and P-8.

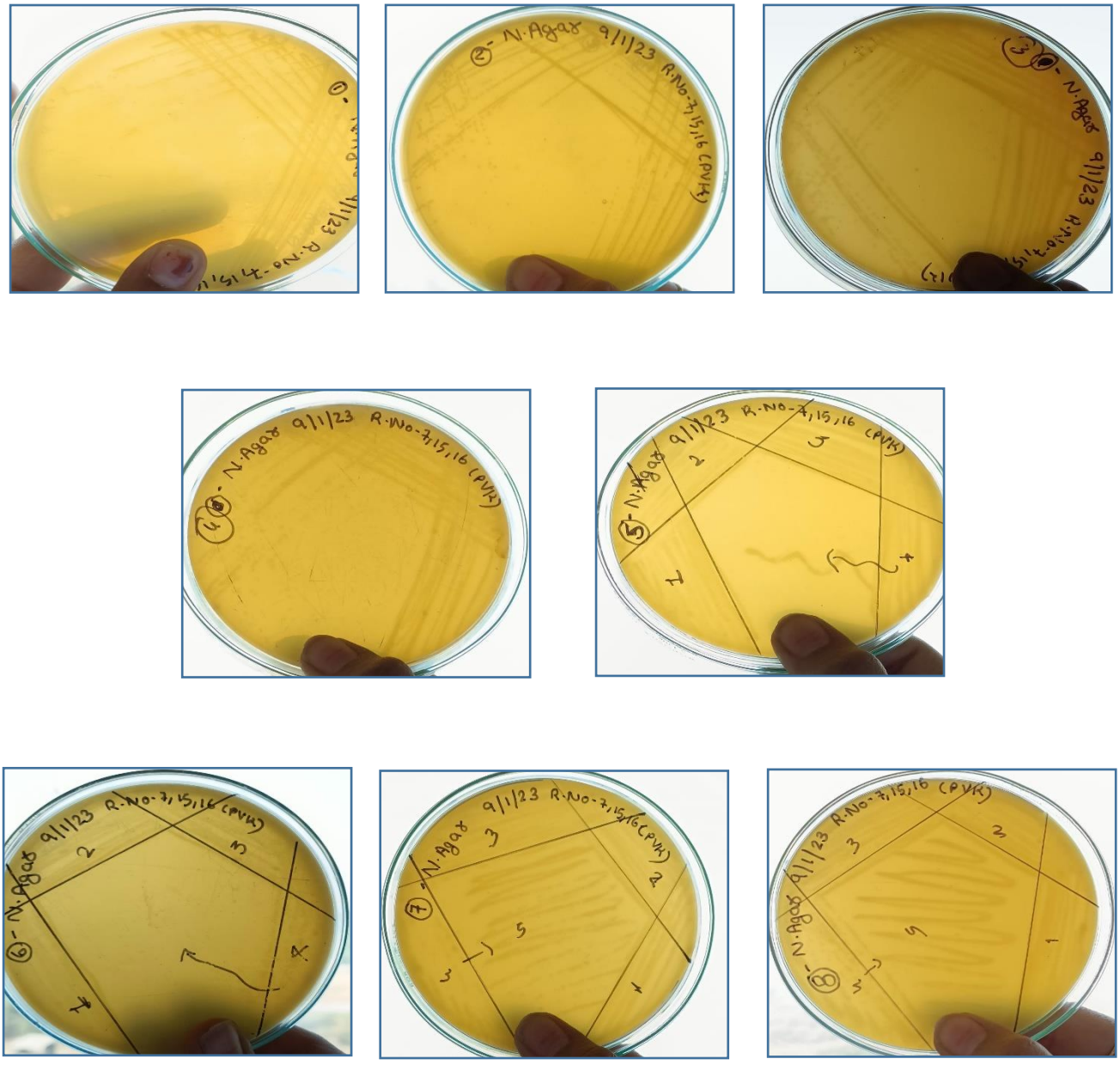
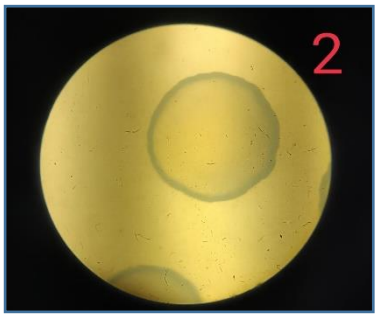


Fig-10 Isolation of colony

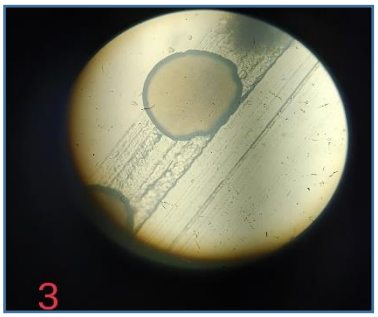
5.2 Colony characteristics

Culture No.	Size	Shape	Margin	Elevation	Surface	Consistency	Pigment	Opacity
P-2	Small	Circular	Undulate	Flat	Smooth	Moist	Yellowish	Translucent
P-3	Small	Circular	Entire	Flat	Smooth	Moist	Yellowish	Translucent
P-4	Small	Circular	Entire	Flat	Smooth	Moist	Yellowish	Translucent
P-8	Small	Circular	Entire	Flat	Smooth	Moist	Yellowish	Translucent

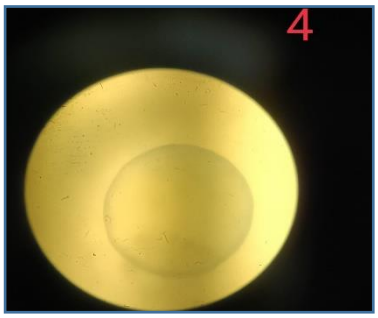
Table-1 Colony Characteristics



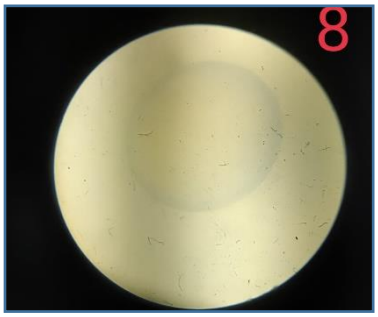
Culture P-2



Culture P-3



Culture P-4



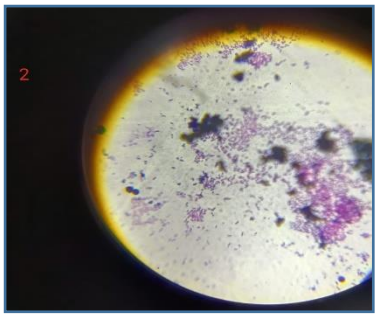
Culture P-8

Fig-11 Microscopic view of colony

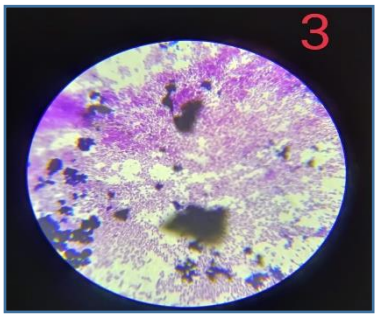
5.3 Morphological characteristics

Culture No	Size	Shape	Arrangement	Gram Reaction
P-2	Small	Short rod	Single, Pair, Clusters	Gram positive
P-3	Small	Short rod & Bacillus	Clusters	Gram positive
P-4	Small	Short rod	Clusters	Gram negative
P-8	Small	Coccus	Clusters	Gram negative

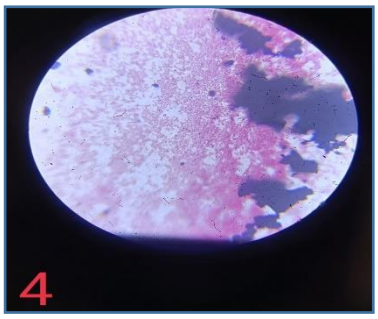
Table-2 Morphological Characteristics



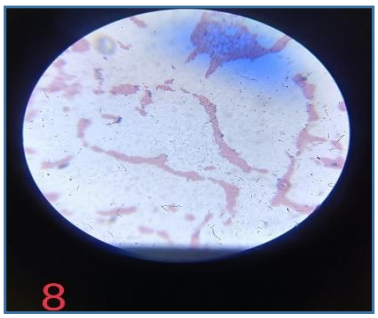
Culture P-2



Culture P-3



Culture P-4



Culture P-8

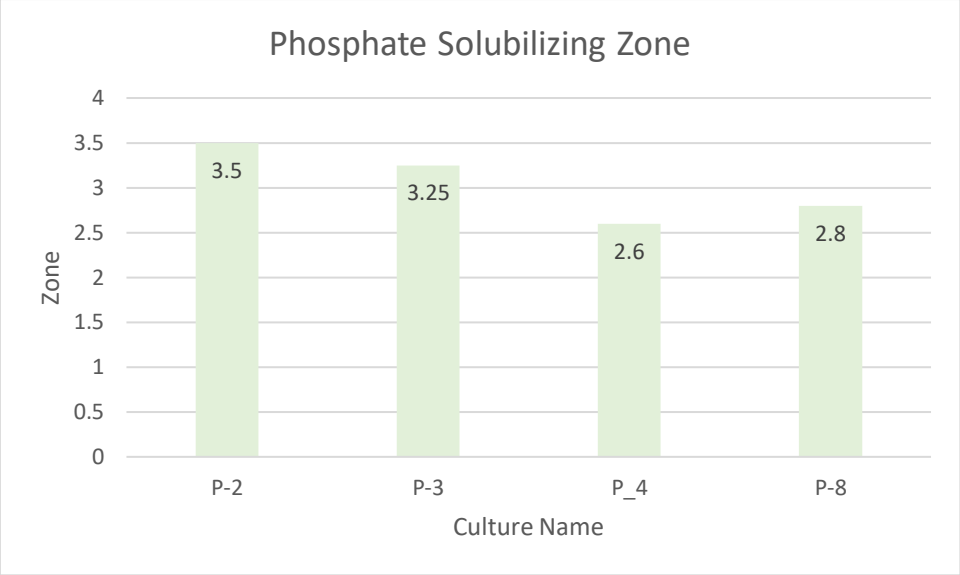
Fig-12 Microscopy of culture

5.4 PGPR results

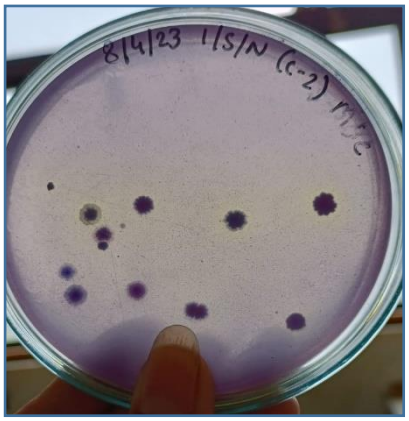
5.4.1 Phosphate solubilisation

Culture Name	Phosphate Solubilisation	Phosphate Solubilizing Zone
P-2	Positive	3.5 cm
P-3	Positive	3.25cm
P-4	Positive	2.6 cm
P-8	Positive	2.8 cm

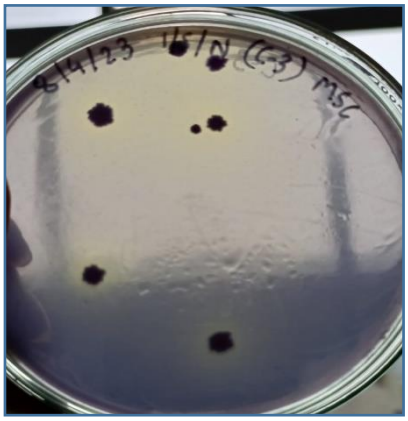
Table-3 Phosphate Solubilisation Zone



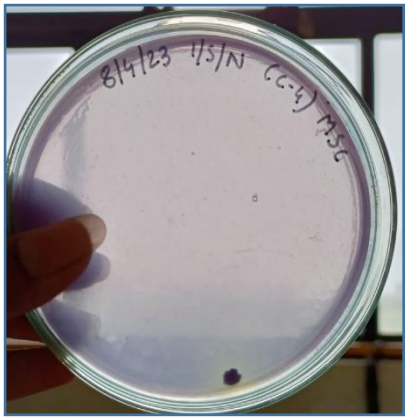
Graph 1- Zone of phosphate solubilisation



P-2



P-3



P-4

P-8

Fig-13 Phosphate Solubilisation Zone

All the 4 cultures, P-2, P-3, P-4 and P-8 are showing positive results for phosphate solubilisation. P-2 has the largest zone of solubilisation while P-4 has lowest zone of solubilisation.

5.4.2 Nitrogen fixation

Culture Name	Nitrogen Fixation
P-2	Negative
P-3	Negative
P-4	Negative
P-8	Negative

Table-4 Nitrogen Fixation

P-2 & P-4

P-3 & P-8

Fig-14 Nitrogen Fixation

As we have isolated our culture from phosphate solubilizing bacteria, all the four cultures are showing negative results for nitrogen fixation.

5.4.3 Ammonia production

Culture Name	Ammonia Production
P-2	Positive
P-3	Positive
P-4	Positive
P-8	Positive

Table-5 Ammonia Production

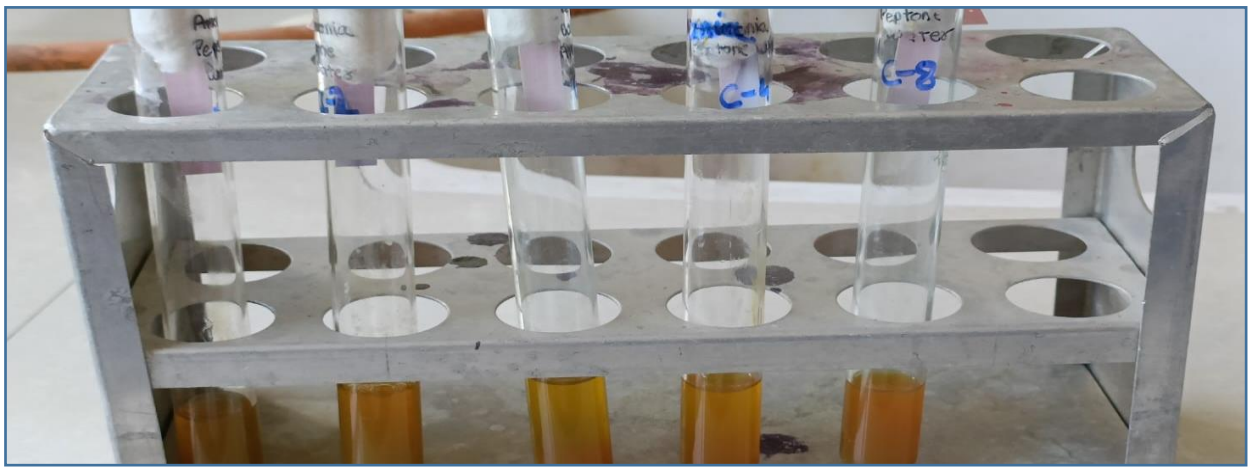
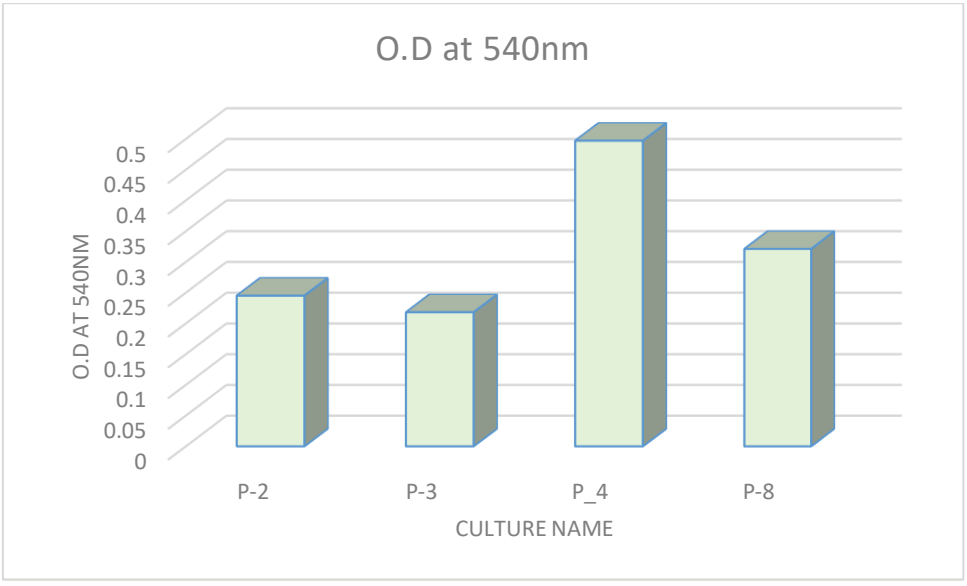


Fig-15 Colour Change Observation of Ammonia Test



Graph-2 Quantitative Estimation of Ammonia

On performing Ammonia production test of our isolates, all the four isolates showed positive result for qualitative estimation of ammonia it means the red litmus paper turned blue and the colour of broth changed to yellow-dark brown. In quantitative estimation we observed that P-4 culture produced highest amount of produced ammonia while P-3 culture produced lowest amount of produced ammonia.

5.4.4 Hydrogen cyanide

Culture Name	Hydrogen Cyanide
P-2	Negative
P-3	Negative
P-4	Negative
P-8	Negative

Table-6 Hydrogen Cyanide

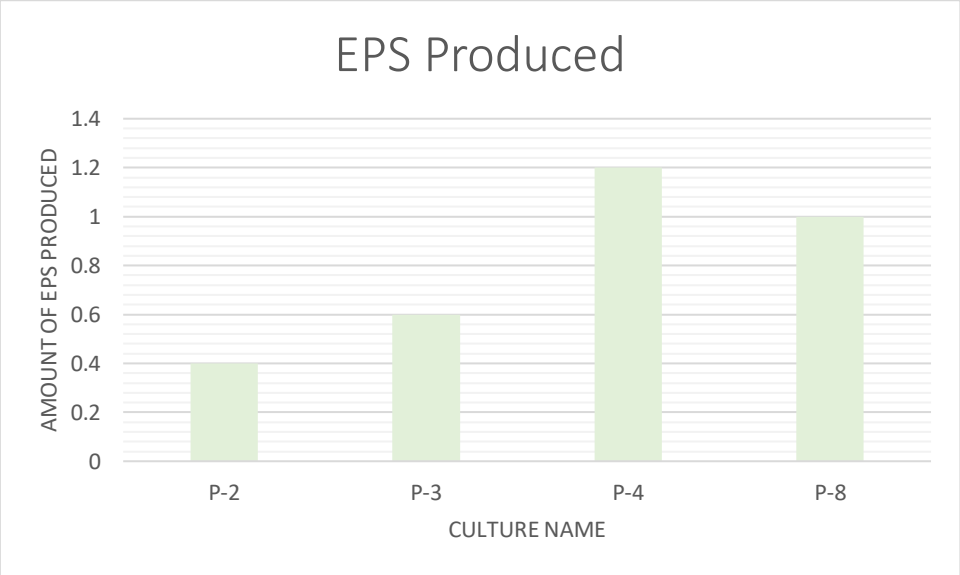
Fig-16 Hydrogen Cyanide Slants

All the four isolates P-2, P-3 P-4. And P-8 has shown negative results for hydrogen cyanide test.

5.4.5 EPS production

Culture Name	EPS Production
P-2	Positive
P-3	Positive
P-4	Positive
P-8	Positive

Table-7 EPS Production



Graph-3 Amount of EPS produced

Fig-17 EPS production

On performing EPS production test of our isolates, all the four isolates showed positive result. Culture P-4 produced maximum amount of EPS while culture P-2 produced lowest amount of EPS.

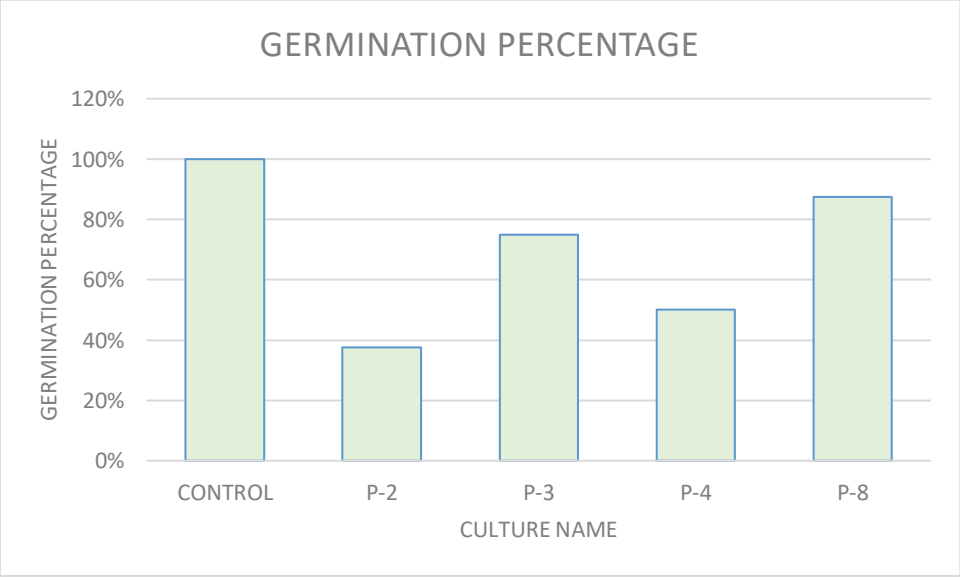
5.4.6 IAA production

Culture Name	EPS Production
P-2	Negative
P-3	Negative
P-4	Negative
P-8	Negative

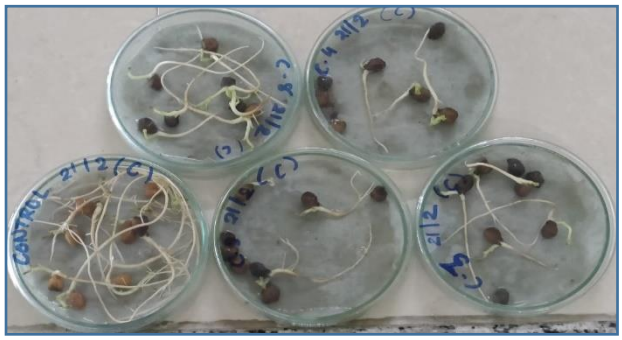
Table-8 IAA Production

All the four cultures are not showing production of tryptophan.

5.5 Seed germination



Graph-4 Germination percentage of 15th day



2nd day result

7th day result

15th day result

Fig-18 Seed Germination

Chickpeas (Desi Chana) were germinated by using culture P-2, P-3, P-4, and P-8. Distill water was used for control. Out of 4 isolates, P-8 culture has shown highest germination percentage while culture P-2 has shown lowest germination percentage.

5.6 Pot study

Fig-19 Image of pot study when seeds were sown

Control

Culture P-2

Culture P-3

Culture P-4

Culture P-8

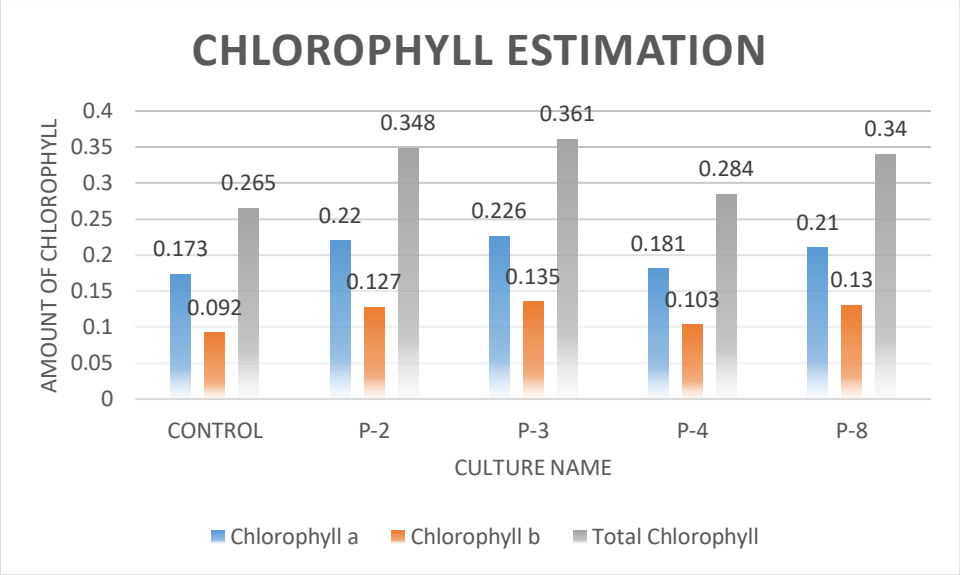
Fig-20 Image of pot on 25th day

Above figures shows the growth of chickpea in 25 days.

5.6.1 Plant growth parameters

- **Chlorophyll estimation**

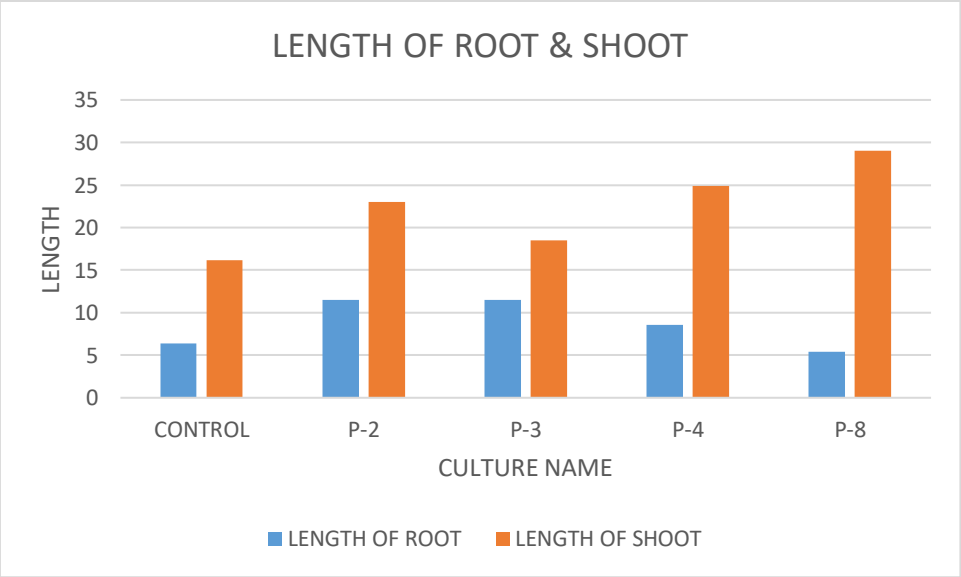
Fig-21 Chlorophyll Estimation



Graph-5 Amount of chlorophyll obtained

We can observe that culture P-3 is giving highest amount of chlorophyll a, chlorophyll b, and total chlorophyll.

- **Length of root and shoot**



Graph-6 Legth of root and shoot

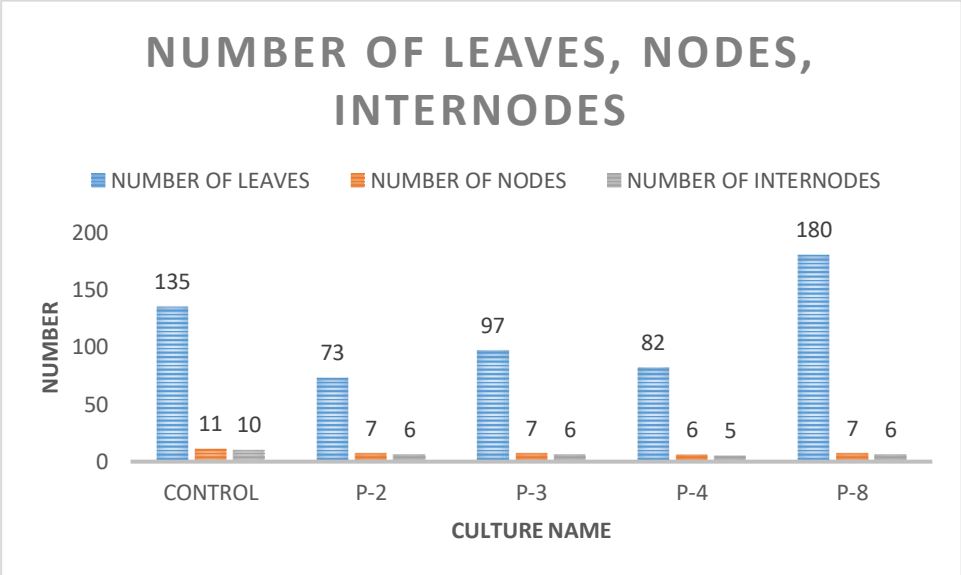
From all the four isolates, culture P-8 is showing largest shoot while culture P-3 is showing smallest shoot. Culture P-2 is showig largest root while culture P-8 is showing smallest root.

- **Fresh and dry weight of root and shoot**

CULTURE NAME	ROOT FRESH WEIGHT	ROOT DRY WEIGHT	SHOOT FRESH WEIGHT	SHOOT DRY WEIGHT
Control	1.012 gm	0.471 gm	0.430 gm	0.140gm
P-2	0.220 gm	0.075 gm	0.370gm	0.165 gm
P-3	0.596 gm	0.262 gm	0.500 gm	0.280 gm
P-4	0.597 gm	0.254 gm	0.370 gm	0.109 gm
P-8	0.654 gm	0.217 gm	0.520 gm	0.229 gm

Table-9 Fresh and dry weight of root and shoot

- **Number of leaves, nodes and internodes**



Graph-7 Influence of biofertilizer on leaves, nodes, internodes

From the above graph, we can observe that highest number of leaves are shown by culture P-8 while number of nodes and internodes are shown almost same by every culture.

CHAPTER-6
CONCLUSION

6. CONCLUSION

All the cultures showed positive results for all the PGPR tests except for nitrogen fixation, hydrogen cyanide and indole acetic acid. Culture P-2 had the highest solubilisation index. It showed solubilisation zone of 3.5 cm. In quantitative estimation of ammonia culture P-4 had shown the highest production. In quantitative estimation of EPS, culture P-4 had shown the highest production of EPS that was 1.2 gm.

In seed germination test, P-8 culture had shown highest germination percentage that was 88% but in compared to control it was 12% less. In chlorophyll estimation, P-3 culture had shown highest amount of total chlorophyll which was 0.095 gm more than control. Culture P-8 had shown maximum shoot length while culture P-2 and P-3 had shown maximum length of root. Culture P-8 had shown maximum number of leaves. Number of nodes and internodes were shown almost equal by all the cultures.

If we select the culture for making a bio-fertilizer on the basis of PGPR we might use Culture P-4. If we select the culture for making a bio-fertilizer on the basis of plant growth parameters then we might use culture P-8.

We can continue this work further in future by (1) Performing field study, (2) By indentifying the cultures, (3) By growing multiple crops at a time.

CHAPTER-7
APPENDIX

7. Appendix

7.1 Media

Pikovskaya agar

Ingredients	Gram/lit
Yeast extract	0.500
Dextrose	10.000
Calcium phosphate	5.000
Ammonium sulphate	0.500
Potassium chloride	0.200
Magnesium sulphate	0.100
Manganese sulphate	0.0001
Ferrous sulphate	0.0001
Agar	15.000

Pikovskaya agar

Ingredients	Gram/lit
Yeast extract	0.500
Dextrose	10.000
Calcium phosphate	5.000
Ammonium sulphate	0.500
Potassium chloride	0.200
Magnesium sulphate	0.100
Manganese sulphate	0.0001
Ferrous sulphate	0.0001

Nutrient agar

Ingredients	Gram/lit
Peptone	5.000
Sodium chloride	5.000
Beef extract	1.000
Yeast extract	2.000
Agar	15.000
pH	7.4±0.2

Nutrient broth

Ingredients	Gram/lit
Peptone	5.000
Sodium chloride	5.000
Beef extract	1.000
Yeast extract	2.000
pH	7.4±0.2

2% Peptone water

Ingredients	Gram/100ml
Peptone	2.000

Luria Bertani broth

Ingredients	Gram/lit
Casein enzymic hydrolysate	10.000
Yeast extract	5.000
Sodium chloride	10.000
pH	7.5±0.2

Ashby's mannitol agar

Ingredients	Gram/lit
Mannitol	20.000
Dipotassium phosphate	0.200
Magnesium sulphate	0.200
Sodium chloride	0.200
Potassium sulphate	0.100
Calcium carbonate	5.000
Agar	15.000

7.2 Reagents

Nessler's reagent

Ingredients	Gram/100ml
Mercuric chloride	10.0
Potassium iodide	7.0
Sodium hydroxide	16.0
Water (ammonia free)	100.0
pH	13.2±0.05

Salkowaski reagent

Ingredients
0.5M Ferric Chloride
35% Picric acid

CHAPTER-8
BIBLIOGRAPHY

Abdullahi, I N, Isah A D, Chuwang, P. Z. (2012). Effect of biofertilizer application on growth of Vitellaria paradoxa seedlings. *Journal of Research in Environmental Science and Toxicology*, *1*(11), 2315–5698. http://www.interesjournals.org/JREST

Anubrata, P., & Rajendra, D. (2014). *Research Article Vegetable Plants With and Without Carrier Materials*.

Bevivino, A., Sarrocco, S., Dalmastri, C., Tabacchioni, S., Cantale, C., & Chiarini, L. (1998). Characterization of a free-living maize-rhizosphere population of Burkholderia cepacia: Effect of seed treatment on disease suppression and growth promotion of maize. *FEMS Microbiology Ecology*, *27*(3), 225–237. https://doi.org/10.1016/S0168-6496(98)00069-5

Bidyarani, N., Prasanna, R., Babu, S., Hossain, F., & Saxena, A. K. (2016). Enhancement of plant growth and yields in Chickpea (Cicer arietinum L.) through novel cyanobacterial and biofilmed inoculants. *Microbiological Research*, *188–189*, 97–105. https://doi.org/10.1016/j.micres.2016.04.005

Burk, D., Lineweaver, H., & Horner, C. K. (1934). The Specific Influence of Acidity on the Mechanism of Nitrogen Fixation by Azotobacter. *Journal of Bacteriology*, *27*(4), 325–340. https://doi.org/10.1128/jb.27.4.325-340.1934

Dasgupta, D., Ghati, A., & Sarkar, A. (2015). Original Research Article Application of Plant Growth Promoting Rhizobacteria (PGPR) Isolated from the Rhizosphere of Sesbania bispinosa on the Growth of Chickpea (Cicer arietinum L .). *International Journal of Current Microbiology and Applied Sciences*, *4*(5), 1033–1042.

Dasgupta, D., Ghati, A., Sarkar, A., Sengupta, C., & Paul, G. (2015). Original Research Article Application of Plant Growth Promoting Rhizobacteria (PGPR). In *Int.J.Curr.Microbiol.App.Sci* (Vol. 4, Issue 5). http://www.ijcmas.com

DHARMAWAN, I. A. (1990). No 主観的健康感を中心とした在宅高齢者における 健康関連指標に関する共分散構造分析Title. *BUDIDAYA AYAM RAS PETELUR (Gallus Sp.)*, *21*(58), 99–104. https://www.unhcr.org/publications/manuals/4d9352319/unhcr-protection-training-manual-european-border-entry-officials-2-legal.html?query=excom 1989

Ghosh, N. (n.d.). *Promoting Bio-fertilizers in Indian Agriculture*. 1–26.

Gupta, G., Ahirwar, N. K., Gandhi, M., & Gramoday, C. (2015). *Plant Growth Promoting Rhizobacteria (PGPR): Current and Future Prospects Microbial & Biochemical Technology Plant Growth Promoting Rhizobacteria (PGPR): Current and Future Prospects for Development of Sustainable Agriculture*. *January*. https://doi.org/10.4172/1948-5948.1000188

K.B. Singh. (1997). Chickpea (Cicer L .). *Field Crops Research*, *53*, 161–170.

Kamaruzzaman, M. A., Abdullah, S. R. S., Hasan, H. A., Hassan, M., Othman, A. R., & Idris, M. (2020). Characterisation OF Pb-RESISTANT plant growth-promoting rhizobacteria (PGPR) from Scirpus grossus. *Biocatalysis and Agricultural Biotechnology*, *23*. https://doi.org/10.1016/j.bcab.2019.101456

Karnwal, A., & Kumar, V. (2012). INFLUENCE OF PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR) ON THE GROWTH OF CHICKPEA (CICER ARIETINUM L.). In *Annals. Food Science and Technology* (Vol. 13, Issue 1). www.afst.valahia.ro

Kaur, P., Singh, J., & Thakur, R. (2018). *Biocatalysis and Agricultural Biotechnology Comparative pot studies of chitosan and chitosan-metal nanocomposites as nano-agrochemicals against fusarium wilt of chickpea (Cicer arietinum L .). 14*(January), 466–471.

Kawaletz, H., Mölder, I., Annighöfer, P., Terwei, A., Zerbe, S., & Ammer, C. (2014). Pot experiments with woody species - A review. *Forestry*, *87*(4), 482–491. https://doi.org/10.1093/forestry/cpu017

Khan, J. (2019). *Biofertilizer- a Possible Subsitute of Fertilizers in*. *January*. https://doi.org/10.13140/RG.2.2.35401.39524

Kizhedath, A., & Suneetha, V. (2011). Estimation of chlorophyll content in common household medicinal leaves and their utilization to avail health benefits of chlorophyll. *Journal of Pharmacy Research*, *4*(5), 1412–1413. http://jpronline.info/article/view/7233/3729

Kumawat, N. (2018). *Popular Kheti*. *February*.

M. A. Laditi. (2012). Evaluation of microbial inoculants as biofertilizers for the improvement of growth and yield of soybean and maize crops in savanna soils. *African Journal of Agricultural Research*, *7*(3), 405–413. https://doi.org/10.5897/ajar11.904

M., M., R., E., A., B., R., B., & H., A. E. (2017). Effect of plant growth promoting rhizobacterial (PGPR) inoculation on growth in tomato (Solanum Lycopersicum L.) and characterization for direct PGP abilities in Morocco. *International Journal of Environment, Agriculture and Biotechnology*, *2*(2), 590–596. https://doi.org/10.22161/ijeab/2.2.5

Mahanty, T., Bhattacharjee, S., & Goswami, M. (2016). Biofertilizers : a potential approach for sustainable agriculture development Biofertilizers : a potential approach for sustainable agriculture development. *Environmental Science and Pollution Research*, *November*. https://doi.org/10.1007/s11356-016-8104-0

Med, J. H. (2015). *Effect of phosphate solubilizing bacteria on the germination of Cicer arietinum EFFECT OF PHOSPHATE SOLUBILIZING BACTERIA ON THE GERMINATION OF CICER ARIETINUM SEEDS AND SEEDLING GROWTH*. *1*(January 2007), 61–63.

Ohyama, T. (2010). Nitrogen as a major essential element of plants. *Nitrogen Assim. Plants*, *37*(January 2010), 1–17. https://www.researchgate.net/profile/Takuji-Ohyama/publication/234135771_Nitrogen_as_a_major_essential_element_of_plants/links/0c96051ac0b614543a000000/Nitrogen-as-a-major-essential-element-of-plants.pdf

Pindi, P. K. (2012). Liquid Microbial Consortium- A Potential Tool for Sustainable Soil Health. *Journal of Biofertilizers & Biopesticides*, *03*(04), 2191–2199. https://doi.org/10.4172/2155-6202.1000124

Rana, A., Saharan, B., Joshi, M., Prasanna, R., Kumar, K., & Nain, L. (2011). Identification of multi-trait PGPR isolates and evaluating their potential as inoculants for wheat. *Annals of Microbiology*, *61*(4), 893–900. https://doi.org/10.1007/s13213-011-0211-z

Ritika, B., & Utpal, D. (2014). Biofertilizer, a way towards organic agriculture: A review. *African Journal of Microbiology Research*, *8*(24), 2332–2343. https://doi.org/10.5897/ajmr2013.6374

Safonova, Y. A., Ivliev, M. N., & Lemeshkin, A. V. (2018). Chickpea seeds germination rational parameters optimization. *Journal of Physics: Conference Series*, *1015*(3). https://doi.org/10.1088/1742-6596/1015/3/032118

Satinder, K. B. (2012). Shelf-life of Biofertilizers: An Accord between Formulations and Genetics. *Journal of Biofertilizers & Biopesticides*, *03*(05), 1–3. https://doi.org/10.4172/2155-6202.1000e109

Savci, S. (2012). Investigation of Effect of Chemical Fertilizers on Environment. *APCBEE Procedia*, *1*(January), 287–292. https://doi.org/10.1016/j.apcbee.2012.03.047

Shahid, M., Singh, A., Srivastava, M., Sachan, C. P., & Biswas, S. K. (2013). Effect of Seed Treatment on Germination and Vigour in Chickpea. *Trends in Biosciences*, *34*(18), 205–207. www.IndianJournals.com

Sharma, S. B., Sayyed, R. Z., Trivedi, M. H., & Gobi, T. A. (2013). Phosphate solubilizing microbes: Sustainable approach for managing phosphorus deficiency in agricultural soils. *SpringerPlus*, *2*(1), 1–14. https://doi.org/10.1186/2193-1801-2-587

Singh, S., Gupta, G., Khare, E., Behal, K. K., & Arora, N. K. (2014). Phosphate Solubilizing Rhizobia Promote the Growth of Chickpea under Buffering Conditions. *Int.J.Pure App.Biosci*, *2*(5), 97–106. file:///home/ford/Documents/PDF/rfd11211.pdf

Smith, A., & Hussey, M. (2020). American Society for Microbiology: Gram Stain Protocols. *American Society for Microbiology*, *September 2020*, 1–9. www.asmscience.org

Smith, S. E., Smith, F. A., & Jakobsen, I. (2003). Mycorrhizal fungi can dominate phosphate supply to plants irrespective of growth responses. *Plant Physiology*, *133*(1), 16–20. https://doi.org/10.1104/pp.103.024380

Vessey, J. K. (2003). Plant growth promoting rhizobacteria as biofertilizers. *Plant and Soil*, *255*(2), 571–586. https://doi.org/10.1023/A:1026037216893