COMPARATIVE STUDY OF LIGNITE BASED AND SHEEP MANURE BASED PSB BIOINOCULANT

A RESEARCH PROJECT

Submitted by

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UNDER THE GUIDANCE OF

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DEPARTMENT OF MICROBIOLOGY

VIVEKANAND COLLEGE, KOLHAPUR

(AN EMPOWERED AUTONOMOUS INSTITUTE)

YEAR 2024-2025

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Dr.Savita D. Mali
ResearchProjectGuide

Examiner

For
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Place:Kolhapur

Date: 3/5/2024

Ms. Priyanka Bhavake
Ms. Samiksha Sakpal
Mrs. Mubina Momin
Mr. Pranav Patil

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CHAPTER 1.0
INTRODUCTION

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1.0 INTRODUCTION

Fertilizer is a material that is applied to soil or plant tissues to provide nutrients for plant growth. Fertilizers provide essential nutrients that plants need to grow, including nitrogen (N), phosphorus (P), and potassium (K).

Bio fertilizers are living microorganisms or their metabolites that promote plant growth, improve soil fertility, and increase crop yields. These microorganisms, when applied to seeds, plant surfaces, or soil, colonize the rhizosphere or the interior of the plant and promote growth by increasing the availability of primary nutrients like nitrogen and phosphorus. (Vessey, 2015). Bio fertilizers help in fixing atmospheric nitrogen, solubilizing soil phosphorus, and stimulating plant growth through the synthesis of growth-promoting substances.

There are two types of bio fertilizers:

1. Liquid bio fertilizer

2. Solid bio fertilizer

The carrier material is a substance that supports and delivers beneficial microorganisms to the soil or plants. An ideal carrier should be non-toxic, moisture-retentive, pH neutral, and able to support microbial survival over time." (Scuba Rao, 982)

There are many different types of carrier materials. These include organic, inorganic, and synthetic materials.

For example:

1) Peat:-

Peat is a type of soft, organic material rich in organic matter and excellent at retaining moisture. It is a preferred carrier globally. However, its availability in India is very limited. (Kantian, 2002)

2) Lignite:-

A type of soft coal, lignite is abundant in areas such as Neyveli in South India. It offers good moisture retention and is cost-effective.

3) Charcoal:-

Known for its high porosity and ability to support microbial survival, charcoal is commonly used, especially when mixed with soil in a 3:1 ratio.

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4. Vermiculture:-

This mineral expands upon heating and provides a sterile, inert medium with excellent water-holding capacity. (Mishra and Solanki, 2015)

5. Press Mud:-

A by-product of the sugar industry, press mud is rich in organic content and nutrients, making it a valuable carrier material.

6. Farmyard Manure (FYM):-

Readily available and rich in nutrients, FYM supports microbial growth effectively.

7. Soil Mixtures:-

Combining soil with other organic materials can create a suitable carrier, especially when other materials are scarce.

8. Bagasse:-

The fibrous residue from sugarcane processing, bagasse has shown promise as a carrier due to its high organic content and ability to maintain microbial viability over extended periods. (Sundara Rao and Tilak, 1977)

Sheep manure is a valuable organic material commonly used in agriculture to enhance soil fertility and support plant growth. It contains essential macronutrients such as nitrogen, phosphorus, and potassium, which are vital for plant development. In addition to these, sheep manure is rich in organic matter that improves soil structure, water retention, and microbial activity. It also provides important micronutrients like copper, manganese, sulphur, boron, and zinc, which play a crucial role in various physiological functions in plants. The comprehensive nutrient profile of sheep manure makes it an excellent natural fertilizer for sustainable farming practices.

Sheep manure is highly nutritious, non-toxic in nature, and widely available in sufficient quantities. It can be easily ground and readily adheres to seeds, making it an excellent choice as a carrier material. Sheep manure is rich in organic matter and serves as a good carbon source. It enhances microbial multiplication and survival by providing essential nutrients and retaining moisture. Its porous and spongy texture helps in moisture retention and aeration, further supporting microbial activity. Sheep manure is also eco-friendly, easily accessible, cost-effective, and sustainable, making it a practical option for agricultural applications.

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In spite of having many beneficial properties of sheep manure it is overlooked by researchers to use it as supporting material in bio-fertilizer production hence, a project was undertaken to evaluate the value of sheep manure as a carrier in the production of microbial inoculant.

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CHAPTER 2.0
REVIEW OF LITERATUR

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2.0 RIVIEW OF LITERATURE

Fertilizers provide essential nutrients that plants need to grow, including nitrogen (N), phosphorus (P), and potassium (K). Since the Green Revolution, fertilizer use has expanded dramatically to meet global food demands. While they have improved agricultural output, concerns over pollution, soil degradation, and greenhouse gas emissions necessitate a revaluation of their use (Tillman et al., 2002).

Sheep manure is an organic by-product of livestock farming that plays a significant role in sustainable agriculture. It enhances soil fertility, improves soil structure, and contribute to the biological activity of soil ecosystems. Unlike synthetic fertilizers, sheep manure releases nutrients slowly, supporting long-term soil health and reducing environmental risks.

As the agricultural sector moves toward more sustainable and eco-friendly practices, organic fertilizers have gained considerable attention. Among them, sheep manure stands out for its nutrient richness, ease of composting, and lower pathogen load compared to some

Other livestock manures (Gaskell & Smith, 2007). Its application supports soil fertility and promotes plant health, making it a valuable resource in integrated nutrient management.

Sheep manure contains essential macro- and micronutrients such as nitrogen (N), phosphorus (P), and potassium (K), as well as calcium, magnesium, and trace minerals (Rynk et al., 1992). According to Maheshwari et al. (2013), composted sheep manure typically contains about 0.7% N, 0.3% P, and 0.9% K, making it a balanced source of nutrition for many crops.

Sheep manure improves soil structure, increases water retention, and enhances microbial activity. The high organic matter content promotes soil aggregation and reduces erosion risks (Diacono & Montemurro, 2010). Additionally, the manure's microbial load Supports biological nitrogen fixation and decomposition, enriching the soil micro biome.

Carrier materials play a critical role in the formulation of bio fertilizers, serving as a medium for microbial survival, transport, and application to the soil or plant. An ideal carrier should be non-toxic to the microorganisms, readily available, cost-effective, easy to process, and capable of maintaining high microbial viability over extended periods.

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Common carrier materials include:

1. Peat

Peat has traditionally been used as a standard carrier due to its high organic matter content, good water-holding capacity, and ability to support microbial survival (Bashan et al.

2. Lignite and Charcoal

Lignite and charcoal are valued for their porous structure, which protects microorganisms (Chandra et al., 2005).

3. Vermiculite and Perlite

These inorganic mineral carriers are lightweight, chemically inert, and have high porosity, making them suitable for bio fertilizer formulations where aeration is important (Malusá & Vassilev, 2014).

4. Coconut Coir and Sawdust

Agricultural waste products like coconut coir dust and sawdust offer sustainable, biodegradable alternatives. Coconut coir, for example, has shown good compatibility with Azospirillum and Rhizobium species (Kumar et al., 2012).

5.Alginate and Other Polymers

Encapsulation of microorganisms in alginate beads or other biopolymers enhances shelf-life, protects from environmental stress, and facilitates controlled release (Bashan et al., 2014).

6.Soil, Compost, and Other Organic Materials

Local soils and composted materials are sometimes used, particularly in 2014). However, concerns regarding sustainability and the environmental impact of peat extraction have prompted the search for alternatives. Small-scale or traditional bio fertilizer production, although they may present challenges related to contamination and variability (Vesey, 2003).

The selection of a suitable carrier material depends on the target microbial species, intended application method, and the agro-climatic conditions of the deployment site.

Although sheep manure has several beneficial properties, it has not been widely studied as a carrier material for bio fertilizer production. Therefore, this study was conducted to evaluate the potential of sheep manure as a carrier for microbial inoculants.

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CHAPTER 3.0

METHODS AND MATERIAL

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3.0 METHODS AND MATEIRAL

3.1 Collection of sheep manure

Sheep manure sample was collected from sheep farm of Kolhapur district. The sheep manure was collected in sterile polythene bags, bags were packed and immediately brought to the laboratory.

Fig.1 Sheep manure

3.2 isolation of bacterial isolates from sheep manure on the basis of phosphate solubilization ability

A) Serial dilution

B) Enrichment

A) Serial dilution

Collected sheep manure was converted into fine powder using mortar and pestle. The powdered sheep manure was then serially diluted using sterile distilled water blank (10^1, 10^2, 10^3, 10^4, 10^5, 10^6, 10^7, 10^8, 10^9, 10^{10}). Each dilution was spread on sterile KB agar plates.

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KB agar composition:-

For 100 ml

Component	Amount
Glucose	1.0 gm
KH2PO4	5.0 ml
CaCl2	10.0 ml
Agar	2.0 gm
Soil extract	100 ml
PH	7

All plates were labelled properly and incubated at room temperature for 24 to 72 hrs. After incubation plates were observed for bacterial colonies showing clear zone around the colony. The selected colonies were stored on nutrient agar slant and all culture were coded as isolate A1, B1, ……, Y1, Z1, A2 B2, ……., Y2, Z2.

B) Enrichment

Collected sheep manure was powdered and inoculated into PK broth. The flask was labelled properly incubated on sterile KB agar plates. The cultures showing clear zone were labelled as above.

3.3 Selection of potent phosphate solubilizing bacterial strain

All isolated strains by serial dilution technique and enrichment technique were studied strain for Gram staining characteristics. The impure cultures were purified by repeated transfer on sterile nutrient agar plate. Finally, all purified cultures were studied for phosphate solubilisation ability. For this equal amount of culture was spot inoculated on sterile KB agar plates. Plates were labelled properly and incubated at room temperature for 3 days. After incubation diameter of colony and diameter of clear zone were measured and solubilization index of phosphate solubilization of each isolate was calculated with the help of following formula.

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Formula:-

SI index = B/A

B= Colony zone diameter

A= Colony diameter

3.4 Identification of potent isolates

On the basis of phosphate solubilisation potent isolates were identified.

3.4.1 Identification by morphological characters

Potent isolates labelled as A1 and F2 were Gram stained to study their morphological characters. The motility of both cultures was studied by hanging drop method. This spore development ability of culture was detected by Dorner's staining method.

3.4.2 Sugar fermentation test

Utilization of different sugars such as Lactose, Maltose, Sucrose, Arabinose, Xylose, Mannitol and Glucose were tested by inoculating fresh culture into Peptone water broth medium containg sugar PH indicator and inverted Durham's tube. Sugar fermentation was detected by change in colour of medium

3.4.2.2 H2S production

Each culture was inoculated into peptone broth medium containing lead acetate paper. After incubation H_2S production was detected by blacking of lead acetate paper

3.4.2.3 Indole production

Peptone broth inoculated cultures after incubation was added with xylene and convex reagent to detect production of Indole.

3.4.2.4 Gelatinase activity

The culture were streaked on sterile Gelatine agar plate. After incubation Gelatinase activity was identified by addition of Frazier's reagent.

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3.4.2.5 Catalase test

A loopful fresh culture was added into H_2O_2 solution to observed catalase production ability.

3.4.2.6 Oxidase test

A loopful of fresh culture was placed on Whatman filter paper, soaked in oxidase reagent, N, N tetramethyl phenylenediamine dihydrochoride to identify oxidase production ability.

3.5 study of biofilm production

Two methods

1) Tube method

2) Congo red agar method

1) Tube method

A loopful of fresh A1 and F2 culture was introduced into a test tube containing 3ml of Nutrient broth supplement with 1% glucose, The tube was incubated at room temperature for 24 hrs. Following incubation. The broth culture was discarded and the tube was rinsed with saline. It was air-dried and stained with 3ml of 0.1% crystal violet at room temperature for 15 min. Excess stain was dried in an inverted position. The presence of violet coloured film on the walls and bottom of the tube indicated biofilm formation. To quantify the biofilm, the bound crystal violet was solubilized using ethanol and the OD of the violet solution was measured at 595nm.

2) Congo red agar method

Congo red agar (Nutrient agar supplemented with 5% glucose and 0.08% congo red indicator) is used. The medium is prepared in two parts (1) Nutrient agar with glucose and (2) A concentrated aqueous solution of congo red. Both components are sterilized separately by autoclaving and then mixed at 55oc before plate preparation. A fresh culture of A1 and F2 is spot inoculated onto the surface of the medium. The plate was then incubated at room temperature for 24hrs. The appearance of black colored colonies with a dry crystalline consistency biofilm production by the organisms.

3.6 Study of auxiliary characteristics of potent isolates

3.6.1 Study of nitrogen fixation ability

Plates of Ashby's mannitol agar were prepared. The potent isolates A1 and F2 were streaked on Ashby's agar medium and plates were labelled properly and kept for incubation for 24 hrs at room temperature to check ability of nitrogen fixation.

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3.6.2 Study of IAA production ability

3.6.2.1 Qualitative study

A nutrient broth with tryptophan was prepared and sterilized. A loopful A1 and F2 was inoculated into separate nutrient broth with tryptophan media. Flasks were labelled properly and incubated at room temperature. Everyday 5ml of broth was removed from each flask and centrifuged at 5000 rpm for 20 min. The supernatant was collected and was added with orthophosphoric acid and Salkowski reagent. The tubes were incubated at room temperature in dark for half an hour. The development of pink colour in the tubes indicate IAA production.

3.6.2.2 Quantitative study

IAA produced by isolates A1 and F2 was estimated with standard graph of IAA (Table 1)

Table 1:- Standard graph of IAA

Sr. No	Standard IAA (ml)	D/W	Concentration of IAA (µg/ml	Orthophosphoric acid (ml)	Salkowaski reagent (ml)	
1	0.2	0.8	20	2	2	Incubation at room temperature for 15 min in dark
2	0.4	0.6	40	2	2	
3	0.6	0.4	60	2	2	
4	0.8	0.2	80	2	2	
5	1.0	-	100	2	2	

3.6.3 Study of enzyme activity

3.6.3.1 Study of amylase activity (starch hydrolysis)

Amylase activity of isolate Al and F2 was studied using starch agar medium. Bacterial isolates A1 and F2 were streaked by cross streaking method on starch agar medium. The plates were incubated at room temperature for 24hrs. After incubation the plates were flooded with iodine solution to observe amylase activity.

3.6.3.2 Study of proteases activity

Caseinase activity of isolate Al and isolate F2 was studied by streaked by cross streaking isolates on milk agar medium. The plates were incubated at room temperature for 24hrs. Plates were observed for development of clear zone around growth

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3.5.4 Study of ammonia production

Peptone water was prepared inoculated with isolate A1 & F2 separately. The tubes were labelled properly and inoculated at RT for 24 hrs. After incubation Nessler's reagent added to detect ammonia production

Nessler's reagent composition :–

Component	Amount
Potassium iodide	50.0 gm
Ammonia free distilled water	35.0 ml
Mercuric chloride solution	35.0 ml
Potassium hydroxide	400 ml
Distilled water	530 ml

3.7 Study of Salt tolereance ability

3.7.1Study of NaCl tolerance ability

Nutrient broth tubes with varying NaCl concentrations (1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, and 10%) were prepared. A loopful of a fresh suspension of isolates A1& F2 was inoculated into each concentration of NaCl separately. The tubes were incubated at room temperature for 24 hours. After incubation, the tubes were examined for growth, indicated by turbidity.

3.7.2 Study of KCl tolerance ability

Nutrient broth tubes with different KCl concentrations (1%, 2%, 3%, 4%, 3%, 6%, 75%, 8%, 9%, and 10%) were prepared. Isolates Al and F2 were inoculated into each concentration and incubated at room temperature for 24 hours. After incubation, the tubes were examined for growth.

3.7.3 Study of Na_2CO_3 tolerance ability

Nutrient broth tubes with different sodium carbonate concentrations (0.1%, 0.3%, 0.5%, 0.7%, 0.9%, and 1%) were prepared. Isolates A1 and F2 were inoculated into each concentration and incubated room temperature for 24 hours. After incubation, the tubes were examined for growth.

3.8 Study of antibiotic sensitivity

The bacterial isolates A1 & F2 were evaluated for antibiotic sensitivity. Nutrient agar plates were prepared, after solidification of agar medium 0.1 ml suspension of each isolate was spread on NA media in aseptic condition. Discs of *Clindamycin, Teicoplanin, Lomefloxacin, Moxifloxacin, Ampicillin, Sulbactam, Cefaclor, Cefadroxil, Roxithromycin, Clarithromycin,*

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linocomycin, Sparfloxacin, Gemifloxiacin, Linezolid and vancomycin were placed over seeded NA plates. Plates were kept for incubation for 24 hrs. at room temperature and after incubation plates were observed for inhibitory zone. The diameter of inhibition zone for each antibiotic was measured.

3.9 Study of Seed germination

3.9.1 Study of Mungbean seed germination

3.9.1.1 Uninoculated seeds

To study seed germination, moist cotton or tissue paper was placed in petri dish. 5ml of distilled water and 20 seeds of *Vigna radiata* (Mungbean) were taken. The seeds were surface sterilized using alcohol, then rinsed and transferred them into petri dish. The setup was kept in a warm place and a few drops of water was sprinkled daily to maintain moisture. The seeds were observed daily for signs of swelling, splitting of the seed coat and the emergence of the root shoot. Record the changes.

3.9.1.2 Seeds inoculated with culture A1

To study seed germination, moist cotton or tissue paper was placed in petri dish. Culture of A1 and 20 seeds of *Vigna radiata* (commonly known as Mungbean) were taken. The seeds were surface sterilized using alcohol, then rinsed and transferred them into petri dish. The setup was kept in a warm place and a few drops of water was sprinkled daily to maintain moisture. The seeds were observed daily for signs of swelling, splitting of the seed coat and the emergence of the root shoot. Record the changes.

3.9.1.2 Seeds inoculated with culture F2

To study seed germination, moist cotton or tissue paper was placed in petri dish. Culture of F2 and 20 seeds of *Vegana radiata* (commonly known as Mungbean) were taken. The seeds were surface sterilized using alcohol, then rinsed and transferred them into petri dish. The setup was kept in a warm place and a few drops of water was sprinkled daily to maintain moisture. The seeds were observed daily for signs of swelling, splitting of the seed coat and the emergence of the root shoot. Record the changes.

3.9.1.2 Seeds inoculated with culture A1 and F2

To study seed germination, moist cotton or tissue paper was placed in petri dish. Culture of A1 and F2 and 20 seeds of Vegan radiate (commonly known as Mungbean) were taken. The

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Seeds were surface sterilized using alcohol, then rinsed and transferred them into petri dish. The setup was kept in a warm place and a few drops of water was sprinkled daily to maintain moisture. The seeds were observed daily for signs of swelling, splitting of the seed coat and the emergence of the root shoot. Record the changes.

3.9.2 Study of Chickpea seed germination

3.9.2 Inoculated seeds

To study seed germination, moist cotton or tissue paper was placed in petri dish. 5ml of distilled water and 20 seeds of *Cicer arietinum* (commonly known as Chickpea) were taken. The seeds were surface sterilized using alcohol, then rinsed and transferred them into petri dish. The setup was kept in a warm place and a few drops of water was sprinkled daily to maintain moisture. The seeds were observed daily for signs of swelling, splitting of the seed coat and the emergence of the root shoot. Record the changes.

3.9.1.2 Seeds inoculated with culture of A1 and F2

To study seed germination, moist cotton or tissue paper was placed in petri dish. Nutrient broth containing culture of A1 and 20 seeds of *Cicer arietinum* (commonly known as Chickpea) were taken. The seeds were surface sterilized using alcohol, then rinsed and transferred them into petri dish. The setup was kept in a warm place and a few drops of water was sprinkled daily to maintain moisture. The seeds were observed daily for signs of swelling, splitting of the seed coat and the emergence of the root shoot. Record the changes.

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daily to maintain moisture. The seeds were observed daily for signs of swelling, splitting of the seed coat and the emergence of the root shoot. Record the changes.

3.10 Production of bio inoculant

3.10.1 Production of lignite based bio inoculant

3.10.1.1 Culture of A1 and F2+ 2gm lignite

The nutrient broth was prepared by adding 2 mL of microbial cultures A1 and F2 into a beaker containing nutrient broth. The mixture was incubated at room temperature for 24 hours. On the second day, 2 gm of lignite was weighed and transferred into a separate beaker. The microbial culture mixture (A1 and F2) was added to the beaker containing the sheep manure and mixed thoroughly using a sterile glass rod to ensure even distribution.

3.10.2. Production of sheep manure bio inoculant

3.10.2.1 Liquid bio fertilizer with sheep manure extract

Two grams of sheep manure was weighed and added to 10 mL of distilled water (D/W). The mixture was allowed to stand for 2–3 days at room temperature. After the incubation period, the mixture was filtered and the resulting liquid extract was used as the bio inoculant carrier.

3.10.2.2 Solid bio inoculant

- **Culture of A1 and F2+ 1gm sheep manure**

The nutrient broth was prepared by adding 2 mL of microbial cultures A1 and F2 into a beaker containing nutrient broth. The mixture was incubated at room temperature for 24 hours. On the second day, 1 gram of finely ground sheep manure was weighed and transferred into a separate beaker. The microbial culture mixture (A1 and F2) was added to the beaker containing the sheep manure and mixed thoroughly using a sterile glass rod to ensure even distribution.

- **Culture of A1 and F2 + 2gm Sheep manure**

The nutrient broth was prepared by adding 2 mL of microbial cultures A1 and F2 into a beaker containing nutrient broth. The mixture was incubated at room temperature for 24 hours. On the second day, 2 gram of finely ground sheep manure was weighed and transferred into a separate beaker. The microbial culture mixture (A1 and F2) was added to the beaker containing the sheep manure and mixed thoroughly using a sterile glass rod to ensure even distribution.

- **Culture of A1 and F2 +1gm Gum+ 1 gm Sheep manure**

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The nutrient broth (NB) had been prepared by adding 2 mL of microbial cultures A1 and F2 to a beaker containing NB. The mixture had been incubated at room temperature for 24 hours. On the second day, a separate beaker had been taken and 1 g of gum had been boiled. Then, 1 g of sheep manure and the previously prepared microbial mixture (A1 and F2 in NB) had been added. The mixture had been thoroughly mixed using a glass rod to ensure uniform consistency.

3.10.3 Mixed (Lignite + sheep manure)

3.10.3.1 Culture of A1 and F2+ 1gm lignite + 1gm sheep manure

The nutrient broth was prepared by adding 2 mL of microbial cultures A1 and F2 into a beaker containing nutrient broth. The mixture was incubated at room temperature for 24 hours. On

The second day, 1 gram of finely ground sheep manure and 1 gm sheep manure was weighed and transferred into a separate beaker. The microbial culture mixture (A1 and F2) was added to the beaker containing the sheep manure and mixed thoroughly using a sterile glass rod to ensure even distribution.

3.10.3.2 Culture of A1 and F2 + 1gm Lignite + 2gm sheep manure

The nutrient broth was prepared by adding 2 mL of microbial cultures A1 and F2 into a beaker containing nutrient broth. The mixture was incubated at room temperature for 24 hours. On the second day, 1 gram of finely ground sheep manure and 2 gm sheep manure was weighed and transferred into a separate beaker. The microbial culture mixture (A1 and F2) was added to the beaker containing the sheep manure and mixed thoroughly using a sterile glass rod to ensure even distribution.

3.10.3.3. Culture of A1 and F2 + 1gm lignite + 2gm sheep manure + 1 gm Gum

Prepared the nutrient broth (NB) by adding 2 mL of microbial cultures A1 and F2 to a beaker containing NB. Incubated the mixture at room temperature for 24 hours. On the second day, took a separate beaker and added 1 g of gum (boiled), then added 1gm lignite and 2 g sheep manure along with the previously prepared microbial mixture (A1 and F2 in NB). Mixed thoroughly using a glass rod to ensure uniform consistency.

3.11 Study of effect of different pot trials

3.11.1 Uninoculated seeds

A volume of 5 mL of distilled water and 20 seeds of *Vigna radiata* (mung bean) were used. The seeds were surface-sterilized using alcohol and subsequently transferred into distilled water. After allowing them to soak for a specific duration, the seeds were sown into pots for further experimentation.

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3.11.2 Nutrient broth + seeds

A volume of 5 mL of distilled water and 20 seeds of *Vigna radiata* (mung bean) were used. The seeds were surface-sterilized using alcohol and subsequently transferred into distilled water. After allowing them to soak for a specific duration, the seeds were sown into pots for further experimentation.

3.11.3 Lignite based bio inoculant

3.11.3.1 Culture of A1 and F2 + 1 gm lignite

Twenty mungbean (*Vigna radiate*) seeds were surface-sterilized using alcohol. Subsequently, the seeds were immersed in a beaker containing 1gm lignite and A1 and F2 contain Nutrient broth. Thoroughly coating of the seeds with the bio inoculant mixture was ensured. After treatment, the seeds were allowed to air-dry briefly and then were sown uniformly in pots containing soil. The pots were monitored daily for seed germination and seedling development.

3.11.4 Sheep manure based bio inoculant

3.11.4.1 Liquid bio fertilizer with sheep manure extract

Surface sterilized 20 mungbean (Vigna *radiate)* seeds using alcohol. Subsequently, the seeds are an extract of sheep manure for bio fertilizer treatment. After thoroughly coating, removed the seeds and sowed them evenly in pots containing soil. Monitored the pots daily to assess seed germination and seedling growth.

3.11.4.2 Solid bio fertilizer

- **Culture of A1 and F2 + 1 gm sheep manure**

Twenty mungbean seeds were surface-sterilized with alcohol and transferred into a beaker containing a pre-prepared mixture of A1 and F2 containing nutrient broth and 1 g of sheep manure (SM). The seeds were thoroughly coated with the mixture and then sown in pots. Daily observations were recorded to evaluate germination rates and seedling development.

- **Culture of A1 and F2 + 2 gm sheep manure**

Twenty mungbean seeds were surface-sterilized with alcohol and transferred into a beaker containing a pre-prepared mixture of A1 and F2 containing nutrient broth and 2 g of sheep manure. The seeds were thoroughly coated with the mixture and then sown in pots. Daily observations were recorded to evaluate germination rates and seedling development.

3.11.5 Mixed bio inoculant (Sheep manure + Lignite)

3.11.5.1 Culture of A1 and F2 + 1gm lignite + 1gm sheep manure

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Twenty seeds of *Vigna radiate* (mungbean) were surface-sterilized using alcohol and transferred into a beaker containing a pre-prepared mixture of formulations A1 and F2. This mixture included nutrient broth (NB), 1 g lignite, and 1 g sheep manure. The seeds were thoroughly coated with the bio inoculant mixture and sown into pots. Daily observations were conducted to assess seed germination and seedling growth performance.

3.11.5.2 Culture of A1 and F2 + 1gm lignite + 2gm sheep manure

Twenty *Vigna radiate* (mungbean) seeds were surface-sterilized using alcohol. Subsequently, the seeds were immersed in a beaker containing 1 gm lignite, 2gm sheep manure and A1 and F2 containg Nutrient broth. Thoroughly coating of the seeds with the bioinoculant mixture was ensured. After treatment, the seeds were allowed to air-dry briefly and then were sown uniformly in pots containing soil. The pots were monitored daily for seed germination and seedling development.

3.12.5.3 Culture of A1 and F2 + 1gm lignite + 2gm sheep manure + 1 gm Gum

Twenty seeds of *Vigna radiata* (mungbean) were surface-sterilized using alcohol and transferred into a beaker containing a pre-prepared mixture of formulations A1 and F2. This mixture included nutrient broth (NB), 1 g lignite, and 1 g sheep manure and 1 gm Gum. The seeds were thoroughly coated with the bio inoculant mixture and sown into pots. Daily observations were conducted to assess seed germination and seedling growth performance.

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CHAPTER 4.0
RESULT AND DISCUSSION

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4.0 RESULT AND DISCUSSION

4.1Isolation1 Isolation of bacterial isolates from sheep manure

A total 4 bacterial colonies were isolated on KB agar plates by serial dilution technique and out of four colonies only one colony was showing clear zone thus indicating phosphate solubilization.

Nearly 28 colonies were obtained by enrichment technique. Out of 28 isolates, 6 colonies were showing phosphate solubilization.

Thus total 7 bacterial isolates having phosphate solubilization ability obtained from sheep manure (Table 2)

Table 2: - Isolation of phosphate solubilizing bacteria from sheep manure

Sr. No.	Isolate name	Phosphate solubilization ability
1	A1	Positive
2	B1	Negative
3	C1	Negative
4	D1	Negative
5	E1	Negative
6	F1	Negative
7	G1	Positive
8	H1	Positive
9	I1	Negative
10	J1	Negative
11	K1	Negative
12	L1	Negative
13	M1	Negative
14	N1	Positive
15	O1	Positive
16	P1	Positive
17	Q1	Positive
18	R1	Negative
19	S1	Positive

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20	T1	Negative
21	U1	Positive
22	V1	Positive
23	W1	Negative
24	X1	Negative
25	Y1	Negative
26	Z1	Negative
27	A2	Negative
28	B2	Negative
29	C2	Negative
30	D2	Negative
31	E2	Positive
32	F2	Positive

4.2 Selection of potent Phosphate solubilizing bacterial strain

Phosphate solubilization ability of all purified isolates (A1, G1, H1, U1, V1, E2, F2) represented in the form of solubilization index is shown in (Table 3)

Table 3: - Solubilization index of all isolates

Sr. no	Isolate name	Diameter of colony zone (mm)			Diameter of clear zone (mm)			SI		
		24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs
1	A1	4.5	4.5	5.3	8.5	10	12	1.88	2.22	2.26
2	G1	8.5	8.5	8	18	20	20	2.11	2.50	2.22
3	H1	6	6	7	10	12	13	1.66	2.0	1.85
4	U1	9	9	10	14	16.5	19.5	1.50	1.83	1.95
5	V1	6.5	7	7.5	7.5	10	11	1.1`5	1.42	1.60
6	E2	5.5	6	6	9	9.5	10	1.63	1.58	1.66
7	F2	6.5	6	7	19	19.5	21	2.90	2.80	3.0

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According to result shown in Table 3 isolate F2 was seen most effective phosphate solubilizer. After F2, isolates A1 was recorded as effective phosphate solubilizer, while V1 showed least solubilization.

4.3 Identification of A1 & F2

4.3.1 Identification by morphological characters

Table 4: - Colony characters of F2 grown on NA plates incubated at room temperature for 24 hrs

size	shape	colour	opacity
2mm	circular	Creamy white	opaque

Margine	Elevation	Surface	Consistency
entire	convex	smooth	moist

Table 5:-Colony characters of A1 grown on NA plates incubated at room temperature for 24 hrs.

Size	Shape	Colour	Opacity
1mm	circular	white	opaque

Margine	Elevation	Surface	Consistency
entire	convex	smooth	moist

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4.3.2 Identification by gram characteristics

Microscopic observation of F2 and A1 revelled that organisms are Gram negative short thick rods arranged signally.

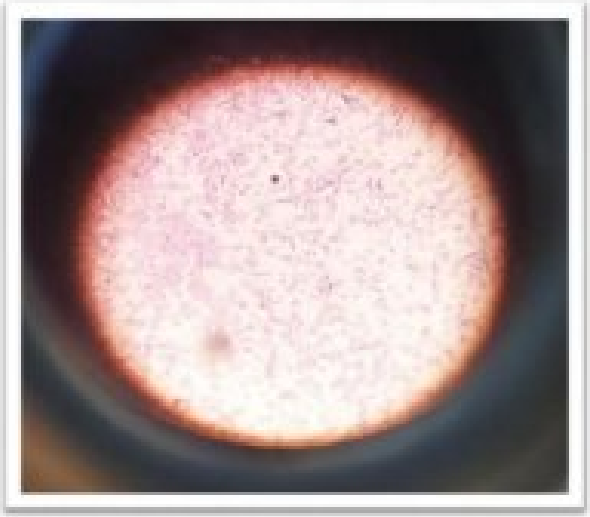

Fig. 2- Gram staining of A1

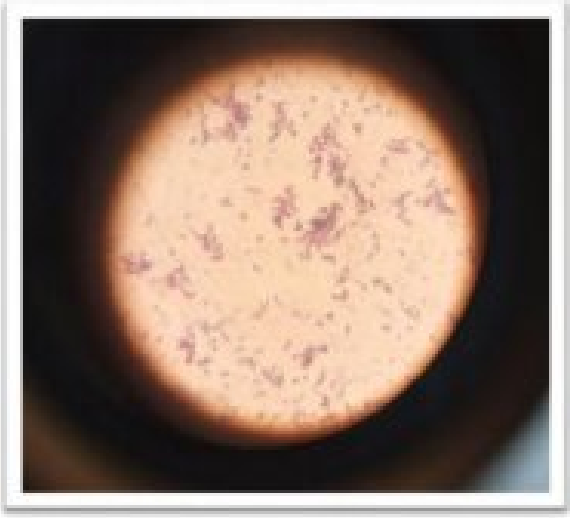

Fig.3- Gram staining of F2

Table 6 Morphological study

Sr. No	Characteristics	Name of isolates	
		F2	A1
1	Gram nature	Gram negative	Gram negative
2	Motility	Motile	Non-motile
3	Spore staining	Sporulating	Non- sporulating

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4.3.3 Identification by biochemical characterization

4.3.3.1 Sugar fermentation test

Table 7: - Sugar fermentations

Sr.no	characteristics	Name of isolates	
		F2	A1
1	Glucose fermentation	Positive	Weakly positive
2	Maltose fermentation	Positive	Weakly positive
3	Mannitol fermentation	Positive	Weakly positive
4	Sucrose fermentation	Positive	Weakly positive
5	Arabinose fermentation	Positive	Weakly positive
6	Xylose fermentation	Positive	Weakly positive
7	Lactose fermentation	Positive	Weakly positive

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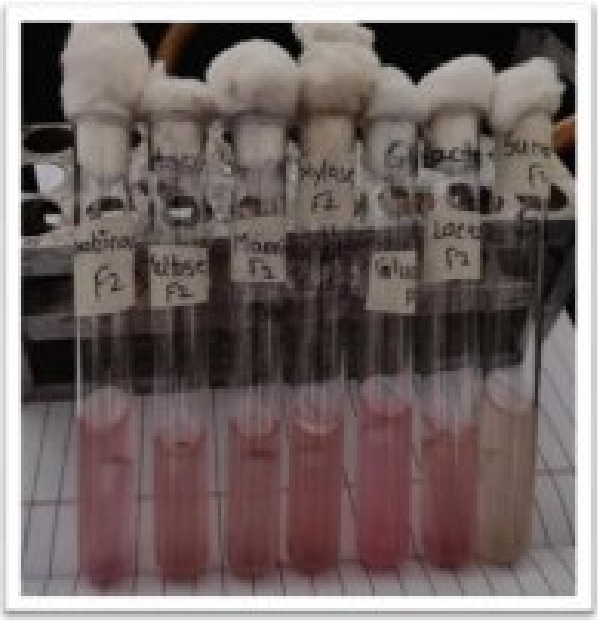

Fig.4 – Biochemical test isolate F2

Fig.5-Biochemical test isolate A1

4.3.3.2 H_2S production

As no blacking of lead acetate paper was seen in F2 &A1 inoculated peptone water tube isolate F2 & A1 have no H_2S production ability.

4.3.3.3 Indole production

Isolate F2 and A1 showed negative indole production activity.

4.3.3.4 Gelatinase activity

After addition of Fraizers reagent in gelatinase agar plate in case of A1 clear zone was observed around the growth. So, gelatinase activity is positive for A1.

I n case of F2 no clear zone was observed thus gelatinase activity is negative.

4.3.3.5 Catalase test

Isolate F2 and A1 showed positive catalase activity.

4.3.3.6 Oxidase test

F2 and A1 showed Isolate positive oxidase test.

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4.4 Study of biofilm production

4.4.1 Test tube method

Table 8:- Biofilm production by test tube method

Parameters	Optical density reading at 595 nm
Control	0.09
Isolate F2	0.09
Isolate A1	0.20
Isolate A1 + Isolate F2	0.11

Isolate F2

OD (Isolate F2) ≤ OD (control) = Non biofilm producer

09 ≤ 0.09 = Biofilm producer

OD (control) ≤ OD (isolate F2) ≤ 2OD (control) =Weak biofilm producer

0.09 ≤ 0.09 ≤ 0.18 = weak biofilm producer

2 OD (control) ≤ OD (isolate F2) ≤ 4 OD (control) = moderate biofilm producer

0.18 ≤ 0.09 ≤ 0.36 = No moderate biofilm producer

4 OD (control) ≤ OD (Isolate F2) =Strong biofilm producer

0.36 ≤ 0.09 = No strong biofilm producer

Thus isolate F2 is a weak biofilm producer

Isolate A1

OD (Isolate A1) ≤ OD (control) = Non biofilm producer

0.20 ≤ 0.09 = Biofilm producer

OD (control) ≤ OD (isolate A1) ≤ 2OD (control) =Weak biofilm producer

0.09 ≤ 0.20 ≤ 0.18 = No weak biofilm producer

2 OD (control) ≤ OD (isolate A1) ≤ 4 OD (control) = moderate biofilm producer

0.1.36 8 ≤ 0.20 ≤ 0= moderate biofilm producer

4 OD (control) ≤ OD (Isolate F2) =Strong biofilm producer

0.36 ≤ 0.20 = No strong biofilm producer

Thus isolate A1 was identified as moderate biofilm producer

Isolate A1+ F2

OD (Isolate A1+ F2) ≤ OD (control) = Non biofilm producer

0.11 ≤ 0.09 = Biofilm producer

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OD (control) ≤ OD (isolate A1+ F2) ≤ 2OD (control) =Weak biofilm producer

0.09 ≤ 0.11 ≤ 0.18 = weak biofilm producer

2 OD (control) ≤ OD (isolate A1+ F2) ≤ 4 OD (control) = moderate biofilm producer

0.18 ≤ 0.11 ≤ 0.36 = no moderate biofilm producer

4 OD (control) ≤ OD (IsolateA1+ F2) =Strong biofilm producer

0.36 ≤ 0.11= No strong biofilm producer

Thus isolate A1 + F2 was seen as weak biofilm producer

Table 9:- Result of biofilm production test of all cultures

Isolates	Biofilm production
A 1	Weak biofilm producer
F2	Moderate biofilm producer
A1 & F2	Weak biofilm producer

Biofilm production in plants is crucial for plant health and growth, playing a vital role in nutrient uptake, protection forms pathogens, and stress resistance. (Table 8, 9 Fig 6).

Fig .6 Biofilm production

4.4.1Congo red agar method

After incubation in Congo red agar plate in case of A1 black colour growth was observed. So, biofilm production activity positive for A1. (Fig7)

In case of F2 no black colour growth was observed thus biofilm production production activity is negative. (Fig.8)

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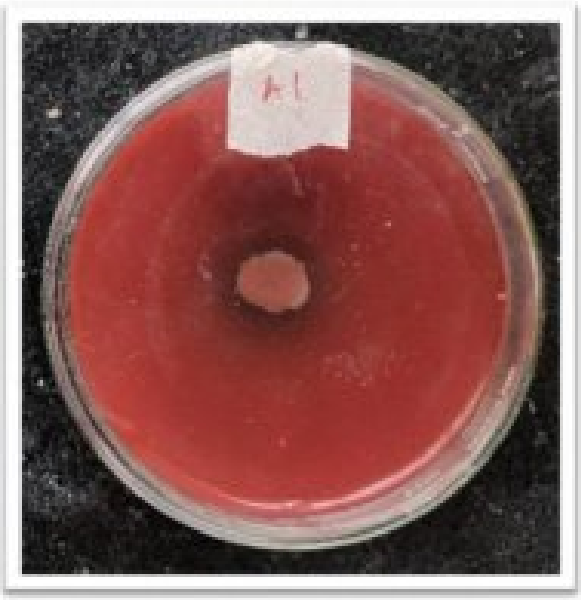

Fig.7-Biofilm production isolate A1

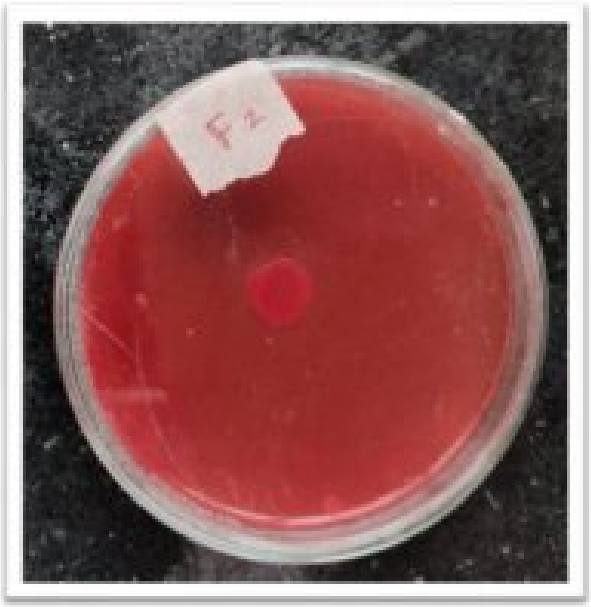

Fig.8- Biofilm production Isolate F2

4.4 Study of auxiliary characteristics of potent isolates

4.4.1 Study of nitrogen fixation ability

On Ashby's mannitol agar medium both F2 & A1 isolates showed growth, thus indicating that both isolates have nitrogen fixation ability.

4.4.2 Study of Indole acetic acid production ability

4.4.2.1 Qualitative study

After incubation of cultures in nutrient broth containing tryptophan and after addition of orthophosphoric acid and Salkowski reagent pink colour was observed in both culture tubes. Thus, it indicated that both F2 and A1 have Indole acetic acid production ability.

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4.4.2.2 Quantitative study

The IAA produced by isolate A1 & F2 was quantified with the help of standard graph (Table10, 11) (Fig. 9).

Table 10: - Standard graph of IAA

Stock IAA – 1000 µg/ml

Conc. Of IAA (µg/ml)	Optical density at 540 nm
Blank	0.0
Unknown	-
20	0.19
40	0.39
60	0.44
80	048
100	0.49

Table 11: - Quantitative estimation of IAA

As per shown in Table 11 A1 was seen producing more quantity of IAA than F2 Table

Sr. No.	isolates	Distilled water (ml)	Orthophosphoric acid	Salkowski reagent	Incubation at room temperature for 15 min in dark	O. D at 530nm		Conc. Of IAA	
						24 hrs	6 days	24 hrs	6 days
1	F2	1	2	2		0.04	0.39	4	44
2	A1	1	2	2		0.04	0.44	4	40

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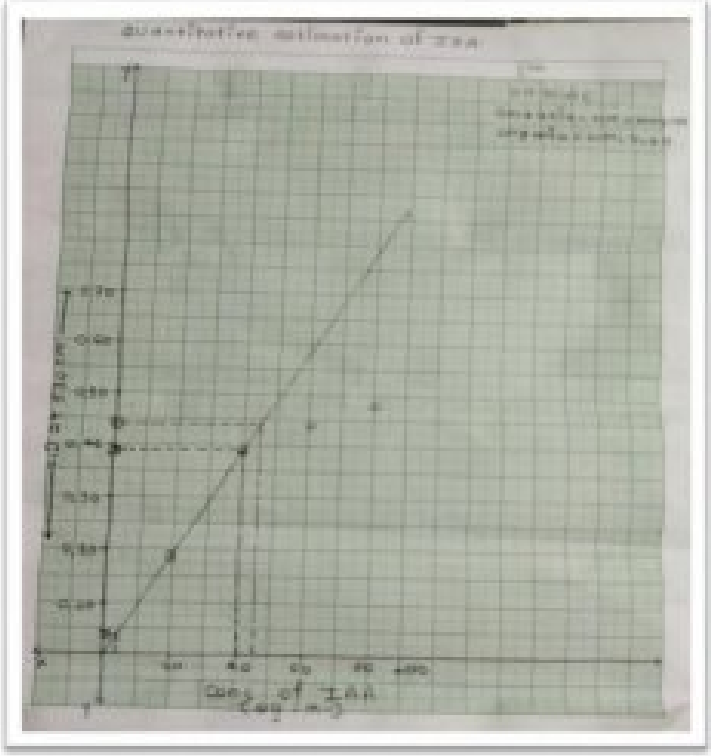

Fig. 9:- Graph showing IAA produced by A1 and F2

4.4.3 Study of ammonia production ability

After addition of Nessler's reagent, production of brown discolouration was observed for both strains indicating production of

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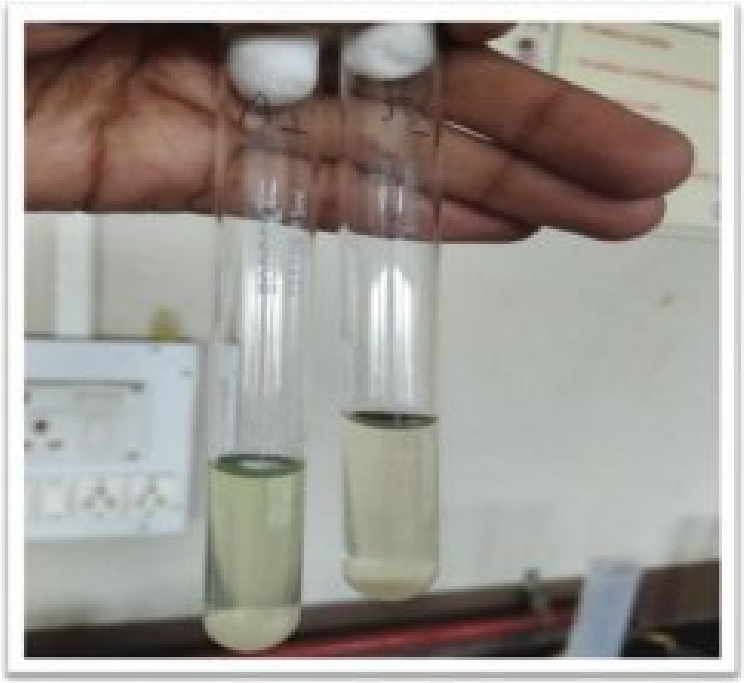
ammonia by both F2 & A1. (Fig.10, 11) The ammonia useful for plant directly or indirectly. Ammonia production by the bacteria helps to



influence plant growth indirectly.

Fig.10 Before addition of Nessler's reagent

Fig.11 After addition of Nessler's reagent

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4.4 Study of enzyme activity

4.4.1 Study of amylase (starch hydrolysis) activity.

Amylase production activity was checked for isolates F2 & A1 by addition of iodine both the isolates showed amylase production ability. Amylase activity is the ability of the enzyme amylase to hydrolyse starches into maltose and other sugars.

4.4.2 Study of protease activity

Protease activity was checked for isolates by using milk agar. After incubation the plate showed no clear zone around the growth, thus indicating that isolates do not carry out production of caseinase. (Fig.12) both the cultures may have other proteolytic activity.

Fig. 12 caseinase activity

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4.5 Study of salt tolerance ability

4.5.1 Study of NaCl tolerance ability

Isolate F2 &A1 showed strong NaCl tolerance ability. Turbidity was observed till 5% & 6% NaCl concentration respectively (Table 12) (Fig. 13,14) This indicates that, these isolates have potential to grow and flourish its life cycle in high saline condition.

Table 12: - Study of NaCl tolerance

Isolates	NaCl concentration (gm %)									
	1	2	3	4	5	6	7	8	9	10
F2	+++	+++	++	++	+	+	-	-	-	-
A1	+++	++	++	++	+	-	-	-	-	-

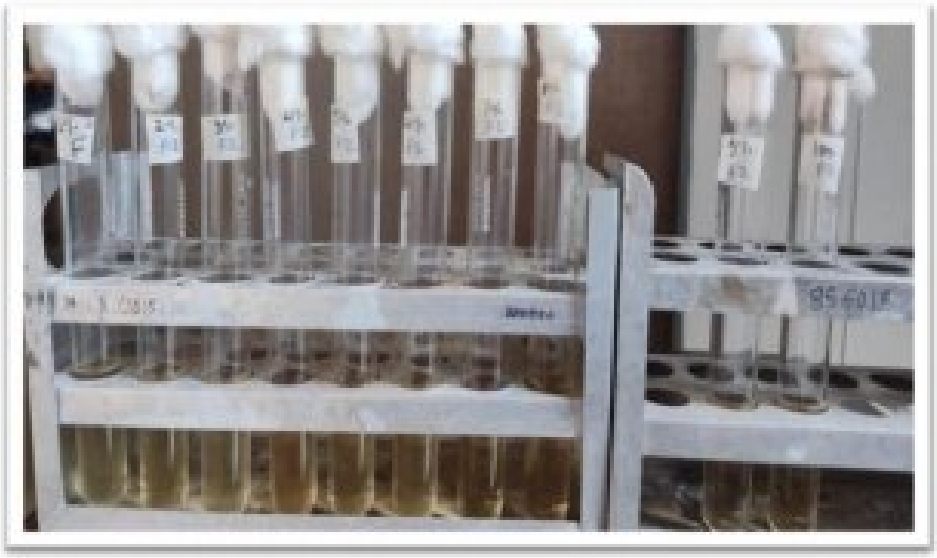

Fig.13-NaCl tolerance of isolate F2

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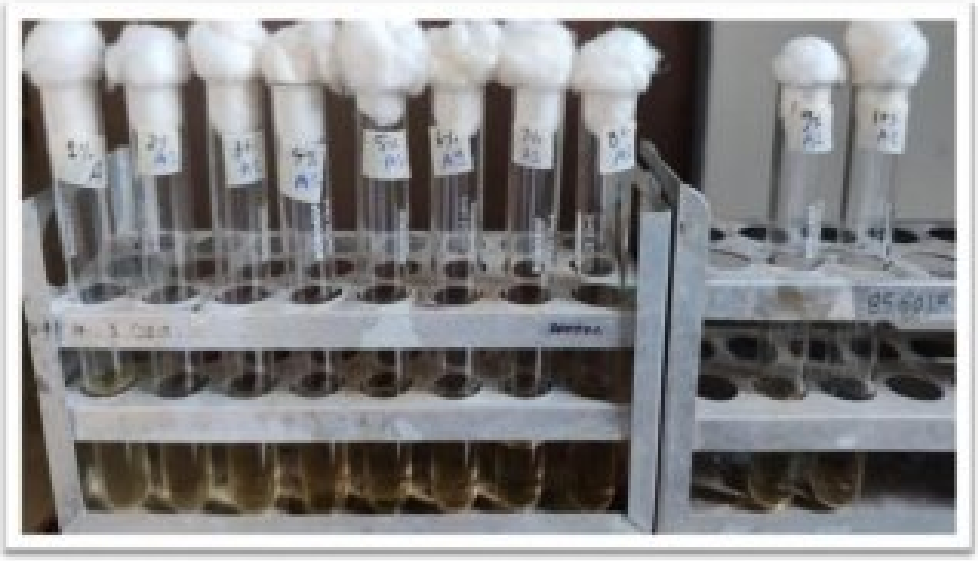

Fig.14- NaCl tolerance of isolate A1

4.5.2 KCl tolerance activity

In case of potassium chloride effect, maximum turbidity was observed of isolate F2 & A1 till 8% & 7% of KCl concentration respectively. This indicates that these isolates are resistant to high KCl concentration, (Table 13) (Fig. 15, 16).

Table 13: - Study of KCl tolerance

Isolates	KCl concentration (gm %)									
	1%	2%	3%	4 %	5 %	6 %	7 %	8 %	9 %	10 %
F2	+++	+++	+++	++	++	+	+	+	-	-
A1	+++	+++	++	++	++	+	+	-	-	-

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Fig.15 KCl tolerance of isolate F2

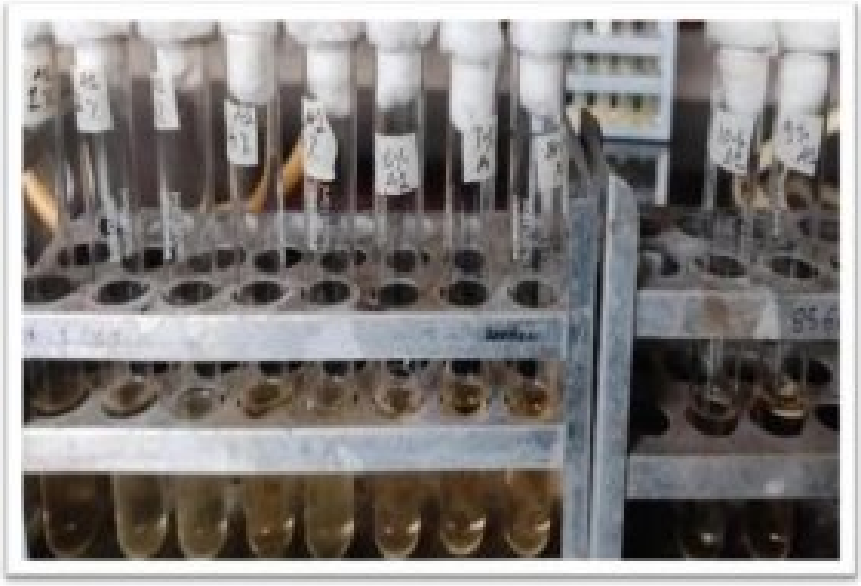

Fig.16 KCl tolerance of isolate A1

4.5.3 Study of Na_2CO_3 tolerance

In case of sodium carbonate tolerance, maximum turbidity was observed of isolate F2 & A1 till 7% & 6% concentration respectively. This indicates that these isolates are resistant to high sodium carbonate concentration, (Table 14) (Fig.17, 18).

Table 14: - Study of Na_2CO_3 tolerance

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Isolates	Na_2CO_3 concentration (Gm %)					
	0.1	03	0.5	0.7	0.9	1
F2	+++	++	+	+	+	-
A1	+++	++	+	+	-	-

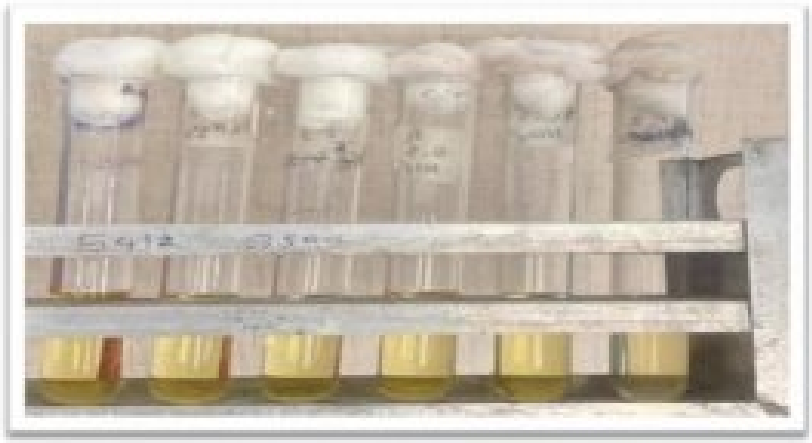

Fig.17- Na_2CO_3 tolerance of isolate F2

Fig.18- 1Na2CO3 tolerance of isolate A1

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4.6 Study of antibiotic sensitivity

Isolate A1 & F2 were studied for antibiotic-sensitivity. In this study, out of 14 antibiotics used isolate F2 showed sensitivity towards nearly 13 antibiotics. Thus, F2 is very safe to use it as bio inoculant (Table 15)

Isolate A1 showed sensitivity towards only 5 antibiotics, out of 14 antibiotics use in the experiment (Table 16).

Table 15: - Antibiotic sensitivity testing of isolate F2

Name of antibiotics	Short forms	Inhibitory zone
Clindamycin	CM/2	3.8
Teicoplanin	TP/30	3.8
Lomefloxacin	LOM/10	4.1
Moxifloxacin	MXF/5	3.5
Ampicillin + sulbactam	SAM	No clear zone
Cefaclor	CEC/30	3.2
Cefadroxil	CFR/30	3
Roxithromycin	RO/30	4
Clarithromycin	CLM/15	4
Lincomycin	LN/15	3.1
Sparfloxacin	SO/5	2.6
Gemifloxacin	GEM/5	3.5
Linezolid	LZ/30	4.1
Vancomycin	VA/30	3.7

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Table 16: - Antibiotic sensitivity testing of isolate A1

Name of antibiotics	Short forms	Inhibitory zone
Clindamycin	CM/2	No clear zone
Teicoplanin	TP/30	2.1
Lomefloxacin	LOM/10	3.45
Moxifloxacin	MXF/5	2.7
Ampicillin + sulbactam	SAM	No clear zone
Cefaclor	CEC/30	No clear zone
Cefadroxil	CFR/30	No clear zone
Roxithromycin	RO/30	No clear zone
Clarithromycin	CLM/15	No clear zone
Lincomycin	LN/15	No clear zone
Sparfloxacin	SO/5	3.5
Gemifloxacin	GEM/5	3.9
Linezolid	LZ/30	No clear zone
Vancomycin	VA/30	No clear zone

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4.8 Study of seed germination

4.8.1 Mung bean (*Vigna radiata*)

Table 17: - Effect of A1, F2 & A1+F2 on germination of mungbean seeds

Seed germination	Isolate A1+F2	Isolate A1	Isolate F2	Control
No. of seed germinated	22	21	20	17
Height (mm)	10,9,8.9,6.8,10.2,4,6,7.8,4.3,5,6.8,3.4,9.8,7.2,5.18.2,3.1,2.1,4.8,5.5,8.7,8.3	8.5,2.1,6.5,3.6,8.4,5.7,6.5,6.6,8.7,5.9,6.8,8.1,1.2,3.5,7.1,8.9,5.5,5.3,4.8,6.7,6.9,7.9	2.7,0.2,4.4,7.9,8.4,3.9,7.7,8.8,1.5,8.9,7.8,2.2,4.8,3.2,3.6,8.9,2.4,1.5,6.9,6.3	7,2,3.4,1.9,6.5,4.1,3.6,2.8,4.5,5.6,3.8,2.2,4.8,3.2,6.6,6.9,7.4
Average height (mm)	6.59	6.43	5.01	4.75

Fig. 19- Seed germination study with A1 +F2 of mungbean seeds

According to results shown in (Table 17), (Fig .19) isolate A1+ F2 in combination were seen most effective for germination of *Vigna radiata* as compared to isolate A1 & isolate F2 & control.

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4.8.2 Chickpea *(Cicer arietinum)*

Table 18:- Effect of A1, F2 & A1+F2 on germination of chick pea seeds

No. of seed germinated	**Isolate A1+F2**	**Isolate A1**	**Isolate F2**	**Control**
Germination seeds	15	14	15	14
Height (mm)	0.2,5.1,2.6,3.6,4.4,0.5,2.5,4.1,5.1,0.6.1.9,2.0,1.9,2.6,4.2,	0.5,2.3,1.2,0.9,0.4,1.3,2.8,1.9,2.2,0.9,1.4,1,1.1,2.8	1.7,0.5,2.9,3.3,2.2,1.9,3.7,3.2,2.3,0.5,2.1,1.4,3.6,1.5,0.5	2.5,1.7,0.8,3.9,4.2,3.1,1.2,2.1,2.5,1.9,0.3,1.6,0.9,2.6
Average height (mm)	2.47	1.47	2.08	1.78

According to results shown in (Table 18), isolate A1+ F2 in combination were seen most effective for germination of *Cicer arietinum* as compared to isolate A1 & isolate F2 & control.

4.9 Production of bio inoculant

4.9.1 Uninoculated Seeds

Table 19: - Pot trials with uninoculated seeds

Days	**1st**	**2nd**	**3rd**
Germination seeds	7	15	17
Height (mm)	-	1.4,1.2,1.1,1.2,0.9,1,0.2,0.8,0.1,0.3,0.4,0.5,0.1,1.3,2.5	19,25,14.7,21,23,13.1,9.8,2.5,11,8.5,16.2,7.2,22.9,19.3,15.1,1.1,0.5
Average height(mm)	-	0.86	13.52

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Fig.20 Pot trials with uninoculated seeds

4.9.2 Nutrient broth + seeds

Table20: - Pot trials with inoculated seeds without carrier (seeds+ NB)

Days	**1st**	**2nd**	**3rd**
Germination seeds	9	10	15
Height (mm)	-	1.6,2,0.8,1,0.6,0.3,1. 91.3,0.1,1.1,	22.4,21.6,19.3,19.5,21,23.9,16.1,11. 5,20.8,6.9,10.5,18.7,12,18.2,14.1
Average height (mm)	-	1.07	17.23

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Fig.21- Pot trials with inoculated seeds without carrier (seeds+ NB)

4.9.2Production of lignite based bioinoculant

4.9.2.1 Culture of A1 and F2 + 2 gm Lignite

Table 21: - Pot trials of lignite based bio inoculant (only 2 gm lignite)

Days	1st	2nd	3rd
Germination seeds	9	10	16
Height (mm)	-	1.5,1.7,1.8,1.9,0.5,0.7,0.3,0.1, 0.9,1	23.2,21.8,12.5,19.8,16.1,7. 5,11.6,18.119.9,5.2,14.5,9. 1,21.3,22,21.6,6.9
Average height (mm)	-	1.04	15.68

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Fig. 22- Pot trials of lignite based bio inoculant (only 2 gm lignite)

4.9.3 Production of sheep manure based bio inoculant

4.9.3.1 Production of sheep manure based solid bio inoculant

Table 22:-Pot trials of sheep manure bio inoculant (only 2 gm sheep manure)

Days	1st	2nd	3rd
Germination seeds	2	14	18
Height (mm)	-	1.2,0.5,2.1,1.0,2.1,0.9, 0.31,0.1,1.1,1.9,1.6,1. 4,2.21.24	16.1,22.1,11.5,14.5,22,21.5,12.5,1 6.1,9.4,8.1,12.6,16.4,15.2,22,20.9, 19,13.2,7.5
Average height (mm)	-	1.24	15.58

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Fig .23- Pot trials of sheep manure bio inoculant (only 2 gm sheep manure)

Table 23:- pot trials of sheep manure based bio inoculant (1 gm gum + 1 gm sheep manure)

Days	1st	2nd	3rd
Germination seeds	2	14	18
Height (mm)	-	1.0,0.5,0.1,0.2,0.9,0.5,0.9, 0.8,0.6,0.1,0.8,0.4,1,0.7	21.8,16.6,19.1,20.5,21,17.518.9,15. 2,9.7,6.5,16.3,12.1,14.5,20.9,15.2,2 0,14,17.3
Average height (mm)	-	0.60	16.50

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Fig.24- pot trials of sheep manure based bio inoculant (1 gm gum +1 gm sheep manure)

4.9.3.2 Liquid Bio fertilizer with sheep manure extract

Table 24: - Pot trials of sheep manure based bio inoculant (sheep manure +D/w)

Days	1st	2nd	3rd
Germination seeds	10	15	18
Height (mm)	-	0.5,0.2,1.2,1.2,1.5,0.5,1.3, 0.9,0.4,1.1,1.5,0.8,1.4,2.1,2.1	23.8,22.1,16.4,21,11.8,19.2,14.2,21.5, 16.2,17.6,11.2,22.3,23.86.3,16.5,23.8, 14.9,12.6,22.1
Average height (mm)	-	1.11	16.09

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Fig.25- Pot trials of sheep manure based bio inoculant (Sheep manure +D/w)

4.9.4 Mixed bio inoculant (Lignite + Sheep manure)

4.9.4.1 Culture of A1 and F2+ 1gm lignite+ 1 gm Sheep manure

Table 25:- pot trials of lignite and sheep manure based bio inoculant (1 gm lignite +1 gm sheep manure)

Day	1st	2nd	3rd
Germination seeds	4	11	16
Height (mm)	-	1,1.4,1,0.1,1.5,0.6,0.8,1,1.4,1.3,0.8	20,20.3,19.8,14.6,9.8,12.6,20,20.5,16.4,11.2,14.8,11.5,20.9,19.8 15.9,19
Average height (mm)	-	0.99	16.69

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Fig.26- pot trials of lignite and sheep manure based bio inoculant (1 gm Lignite +1 gm sheep manure)

4.9.3.2 Culture of A1 and F2+ 1gm lignite+ 2 gm Sheep manure

Table 26: - Pot trials of lignite and sheep manure based bio inoculant (1gm lignite + 2 gm Sheep manure)

Day	1st	2nd	3rd
Germination seeds	9	10	18
Height (mm)	-	2.2,1.4,0.5,1.0,0.8,0.9,2.1,2.2,1.9,0.2	24.7,21,19.8,12.9,22,22.1,16.5,21.1,20.8,21.6,24.2,23.8,15.2,16.7,23.5,22,,14.2,9.6,
Average height (mm)	-	1.32	19.56

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Fig.27- Pot trials of lignite and sheep manure based bio inoculant (1gm lignite+ 2 gm Sheep manure)

4.9.3.2 Culture of A1 and F2+ 1gm lignite+ 1gm Sheep manure + 1 gm Gum

Table 27: - pot trials of lignite and sheep manure based bio inoculants (1gm lignite+ 1gm Sheep manure + 1 gm Gum)

Day	1st	2nd	3rd
Germination seeds	2	14	17
Height (mm)	-	1.2,0.5,2.1,1.0,2.1,0.9,0.3,1,0.1,1.1,1.9,1.6,1.4,2.2	21.9,12.9,16.9,21.7,12.5,11.4,19.5,5.3,16.2,17,20.1,21,21.5,12.5,19.1,11.2,14.3
Average height (mm)	-	1.24	16.17

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Fig. 28- pot trials of lignite and sheep manure based bio inoculants (1gm lignite+ 1gm Sheep manure + 1 gm Gum)

According to the results shown in (Table 19,20,21,22,23,24,25,26,27) and (Fig. 20, 21, 22, 23, 24, 25,26,27,28). 2g of sheep manure + 1g of lignite formulation is revealed the most effective bio-inoculant as compared to other bio-inoculants, making it a useful formulation to produce bio-fertilizer.

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4.9.6 Study of dried seedlings

Table 28: - Study of dried seedlings

Sr.no	Carrier material	Dry Weight of seedlings (gm)
1	2 gm lignite	0.04
2	1 gm lignite+ 1gm sheep manure	0.11
3	1 gm lignite + 2 gm sheep manure	0.04
4	2 gm sheep manure	0.13
5	Control	0.13
6	Control NB + Seeds	0.12
7	Liquid sheep manure based bio fertilizer	0.18
8	1 gm sheep manure +1 gm lignite + 1 gm gum	0.18
9	1 gm sheep manure + 1 gm gum	0.13

According to the results shown in (Table 28), liquid sheep manure-based bio inoculant formulation and 1g of sheep manure + 1g of lignite + 1g of gum arabic formulation were seen the most effective for mug bean as compared to other bio inoculant formulations.

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CHAPTER 5. 0

SUMMARY AND CONCLUSION

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5.0 SUMMARY AND CONCLUSION

Thirty-two isolates were isolated on KB agar plates from sheep manure. Out of 32, twelve isolates (A1, G1, H1, N1, O1, P1, Q1, S1, U1, V1, E1, and F2) showed clear zones around colonies. Only seven isolates, coded as (A1, G1, H1, U1, V1, E2, and F2), and showed measurable clear zones.

Seven isolates were further evaluated for phosphate solubilization. Two isolates, labelled as F2 and G1, showed potent phosphate solubilization ability. Besides phosphate solubilization, these isolates also exhibited nitrogen fixation, IAA production, and ammonia production abilities.

The salt tolerance ability of isolates F2 and G1 indicated that they can survive and thrive under high salt conditions. Thus, these isolates could be useful as bio-inoculants for saline soils.

Sodium hydrogen carbonate was tested to evaluate their potential to survive and thrive under high salt conditions.

Antibiotic sensitivity was analyzed using various antibiotics to test the response of potent isolates. The promising results revealed that the isolates were sensitive to the antibiotics, enabling safe usage of isolates as bio-inoculants.

Seed germination effects were tested using isolates A1 and A2. For this, we used mung bean (*Vigna radiata*) seeds. The isolates were tested both separately and in combination. The combination of isolates A1 and F2 showed more effective germination of *Vigna radiata* compared to individual isolates A1 and F2, and the control.

Seed germination tests were also conducted using chickpea (*Cicer arietinum*) seeds. Isolates A1 and F2 were mixed with chickpea seeds separately as well as in combination. The combination of isolates A1 and F2 showed more effective germination of *Cicer arietinum* compared to individual isolates A1, F2, and the control.

Bioinoculants were produced using different carrier materials mixed with isolates A1 and F2. These tests were conducted in pot trials. Various carrier materials such as lignite, and sheep manure were tested.

Different formulations described below were evaluated

1. Only lignite,
2. lignite + sheep manure,
3. lignite + sheep manure + gum arabic,
4. sheep manure + gum Arabic, and

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5. liquid sheep manure

Only liquid sheep manure-based bio-inoculant formulation and 1 gm sheep manure + 1 gm of lignite + 1 gm of gum Arabic formulation were found to be most effective for mungbean, as compared to other bio-inoculant formulations.

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CHAPTER 6.0
BIBLIOGRAPHY

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6.0 BIBLIOGRAPHY

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