FORMULATION OF CARRIER BASED BACTERIAL INOCULANT USING WASTE TEA POWDER

A RESEARCH PROJECT

SUBMITTED BY

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VIVEKAKNAND COLLEGE, KOLHAPUR

(EMPOWERED AUTONOMOUS)

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This is to certify that **Ms. Suhasi Rahul Kamble** studying in M.Sc. II Microbiology at Vivekanand College, Kolhapur (Empowered Autonomous) have sincerely completed research project work entitled as **"FORMULATION OF CARRIER BASED BACTERIAL INOCULANT USING WASTE TEA POWDER"** prescribed by Vivekanand College, Kolhapur during academic year 2023-24.

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This is to certify that **Mr. Sujay Prakash Parase** studying in M.Sc. II Microbiology at Vivekanand College, Kolhapur (Empowered Autonomous) have sincerely completed research project work entitled as **"FORMULATION OF CARRIER BASED BACTERIAL INOCULANT USING WASTE TEA POWDER"** prescribed by Vivekanand College, Kolhapur during academic year 2023-24.

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Place: Kolhapur

Date: 15/5/24

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CHAPTER 1.0

INTRODUCTION

1.0 INTRODUCTION

Food waste is a major component of organic waste that has widespread issue in global food system. Globally each year approximately one third of the food produced for human consumption is lost or wasted which is approximately 1.3 billion metric tons. Organic material act as valuable source of plant available nutrients e.g. nitrogen (N) , phosphorus (P) , potassium (K) , sulfur (S) and magnesium (Mg) and thereby reduced the need for manufactured fertilizer inputs. The management of food waste varied according to countries. According to data on current food waste treatment in developing nations,the most prevalent food waste treatment method in dumps/ landfilling, wich are used for food wast treatment at a rate of over 90% .

Tea powder is also one component of food waste of organic origin. tea which is prepared from *Camellia sinesis* leaves is one of the world‟s most popular nonalcoholic beverages . Waste tea leaves can be a great source of organic material for garden and compost piles. Tea leaves are high in tannic acid which helps to increase oxygenation and facilitates the growth of a stronger root system. Tea leaves are effective for fruit bearing plants, herbs, and flowering plants. According to the report published by international tea committee global tea consumption exceeded 5.8 million tons.

The immense consumption of tea also generates thousands of kilos of waste tea powder, most of which is tossed in dustbins, but this waste can actually be used as nutrient rich fertilizer.

Tea powder contains nitrogen along with phosphorus and potassium as well as other micronutrients that are beneficial for soil and plants as well. The used tea powder often has herbs such as holy basil, ginger, cardamom and sugar . It‟s important to wash the tea

powder before turning it into a bio fertilizer in order to avoid bad odor and ants. Tea powder decomposes easily in landfills and do not cause harm to environment

Bio fertilizer application to agriculture land can result in changes in soil , physical properties such as water retention , filtration rate , biological properties and crop yield . Biofertilizer may be potential to fufill the demand for the world‟s fertilizer‟s by utilizing food waste while also addressing waste management and nutrient recycling Biofertilizer‟s is the bioconversion process where compelx organic matter is degraded by microorganisms and converted to human-rich fertilizer manure.

The present study was carried out to use the tea powder that is any how wasted especially in urban areas which is not utilized for any purpose and discarded as wet garbage . Tea powder can be a great source of biodegradable garbage but it can make a good source of compost as well . Using of waste tea powder has increased concentration of essential nutrients needed for plant growth and development as compared to the regular soil which is chloride , sulphate , total phosphorus , organic matter , calcium and magnesium . By using waste tea powder plant grow rapidly and there is increment in leaf area , leaf density , height , and germination period and germination frequency of plant . Thus , it also reduces environmental pollution and also gives better yield of crops .

CHAPTER 2.0

REVIEW OF LITERATURE

2.0 REVIEW OF LITERATURE

Biofertilizer are usually carrier based inoculant containing beneficial microorganisms. Incorporation of microorganisms in carrier material enables easy handling, long term storage and high effectiveness of biofertilizers.

In carrier based inoculant, peat, wood charcoal and lignite are used as carriers and these inoculants suffer from poor quality, high contamination and unpredictable field performance. Whereas, tea powder based biofertilizer of good quality hold great promise in agriculture. Tea leaves contain the three big nutrients termed NPK, i.e. nitrogen, phosphorus, and potassium. Used tea leaves not only increase agricultural yields and offer farmers an economical natural fertilizer. It contains special cell protectant or substance that encouraged the formation of resting spores or cyst for longer shelf life and protect the cell against seed toxicity after seed application.

Bio fertilizers are a promising alternative solution to reduce the long-term adverse effects of chemical fertilizers and important for promoting sustainable agriculture. Unfortunately, bio fertilizers have a relatively short shelf life, and microbial effectiveness often decreases during storage and application. Therefore, innovation is needed regarding the formulation of biological fertilizer carriers that have the potential to maintain microbial viability and effectivity during storage. The comprehensive study was carried out using Systematic Literature Review (SLR) method by the search engine to evaluate and assess the current status of solid carrier formulation to improve the viability and effectiveness of bio fertilizers inoculants. The results of a systematic review of scientific literature were obtained from as many as 149 articles from Science Direct and Scopus, and a total of 10 articles were chosen for further review. Several carrier materials have been reported can increase the viability and effectiveness of N-fixing inoculant. Each carrier material provides various

benefits, such as increased microbial shelf life, microbial activity, and plant growth. Some carrier materials have the potential for further development in Indonesia. (Kata Kunci: Bio fertilizer, Indonesia, PRISMA, Shelf life)

Tea leaf biochar is known to increase microbial biomass and enzyme activity (Azeem et al., 2021). Biochar facilitates a suitable habitat for bacteria growth because it is rich in nutrients (C, N). The organic matrix entrapped biofertilizer (OMEB) can increase the microbial activity of A. chroococcum and B. subtilis, which is indicated by an increase in dehydrogenase and phosphatase enzymes secreted by microbes (Kumar et al., 2015).

According to Azeem et al. (2021), tea leaf biochar can improve the viability and stability of microbial cells even after three months of storage. These results were obtained when tea leaves were pyrolyzed at 600oC compared to tea leaves pyrolyzed at 300oC.

CHAPTER 3.0
MATERIAL AND METHODS

3.0 MATERIAL AND METHODS

3.1Isolation and selection of bacterial isolates

3.1.1 Collection of soil sample

Soil samples were collected from banana field of Sangli district and sugarcane field of Kolhapur district. The soil samples were collected in sterile Polythene bags and immediately brought to the laboratory.

Fig ; Sugarcane and banana soil

Serial dilutions of samples were done from 10-1 to 10-6 in the sterile distilled water. 0.1 ml of each dilution was spread on the sterile nutrient agar plates. The plates were labelled properly. These plates were then incubated at room temperature for 24 hrs. After incubation, identification of the bacterial isolates was done by microscopic observation.

3.1.2 Morphological characteristics of bacterial isolates.

Nearly 12 well isolated colonies were selected for morphological studies. Selected colonies were Gram stained. The impure cultures were further streaked on nutrient agar plates for purification. Finally pure cultures were labelled as isolate - 1, isolate - 2, isolate - 3 , isolate - 4, isolate - 5, isolate - 6, isolate - 7, isolate - 8, isolate – 9, isolate - 10, isolate - 11, isolate - 12. The colony characters of all 12 pure isolates were notes down.

3.2 Selection of culture on the basis of phosphate solubalization

Plates were prepared of KB (King`s B) agar medium were prepared , 12 isolates were spot inoculated on the plates and plates were incubated at room temperature for 24 hrs. Plates were then examined for development of clear zone around colonies and observations were noted down .

3.3 Study of nitrogen fixation ability

Plates of Ashby"s Mannitol Agar were prepared. Twelve isolates were spot inoculated on Ashby's agar medium and plates were kept for incubation for 24 hrs. at room temperature, to check ability of nitrogen fixation.

3.4 Identifications of potent isolates

On the bases of phosphate solubalization ability and nitrogen fixation ability, two potent isolates were identified i.e. isolate 1 and isolate 6.

3.4.1 Identification by morphological characters of potent isolates

Potent isolates 1 and 6 were identified by morphological characters for this Gram staining procedure was carried out.

3.4.2 Identification by biochemical characters

Sugar fermentation tests was performed For Isolate 1, Isolate 6. and mixture of 1 and 6. (Different sugars such as Lactose, Maltose, Sucrose, Dextrose, Ribose, Galactose, Mannitol and Glucose were tested). Peptone broth with Andrade"s indicator and Durham"s tubes in it were prepared and loopful of a cultures suspension was inoculated aseptically in sterile tube of peptone broth The inoculated tube was kept for incubation at room temperature for 24 hrs. After incubation tubes were examined for acid (color change) and gas production,

3.5 Study of NaCl (Sodium chloride) tolerance-

Nutrient broth tubes with different NaCl concentrations such as 1%, 2%, 3%, 4%, 5%, 6%, 7% 8%,9%, 10% were prepared.

A loopful of fresh suspension of isolate 1 and isolate 6 was inoculated in each concentration of NaCl tubes and all tubes were incubated at room temperature for 24 hrs. After incubation tubes were examined for growth i.e. turbidity.

3.6 Study of KCl (potassium chloride) tolerance effect -

Nutrient broth tubes of different KCI concentrations such as 1%, 2%, 3%, 4%. 5%, 6%, 7%, 8%, 9%, 10% were prepared.

Isolate 1 and isolate 6 were separately inoculated in each concentration of KCl tubes and tubes were incubated at room temperature for 24 hrs. After incubation tubes were examined for growth.

3.7 Study of sodium carbonate (Na2Co3) tolerance -

The nutrient broth tubes of different sodium carbonate concentrations such as 0.1%, 0.3%, 0.5%, 0.7% , 0.9% , 1% were prepared.

Isolate 1 and isolate 6 were inoculated in each tubes and kept for incubation at room temperature for 24 hours, After incubation tubes were examined for growth.

3.8 Study of sodium hydrogen carbonate (NaHCO3) tolerance

The nutrient broth tubes of sodium hydrogen carbonate concentration of different dilutions such as 0.1%, 0.3%, 0.5%, 0.7% , 0.9% , 1% were prepared.

Isolate 1 and isolate 6 were inoculated in each tubes and kept for incubation at room temperature for 24 hours, After incubation tubes were examined for growth .

3.9 Study of amylase (starch hydrolysis) activity-

Amylase activity of isolate 1 and 6 was studied using starch agar medium .

Bacterial isolates 1, 6 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.

3.10 Study of ammonium production ability-

Ammonia production ability of isolates was studiesd using 4% peptone water. Suspension of Isolate 1 , Isolate 6 and Mixture isolate 1 and 6 were prepared. Ten ml peptone water was taken in each flask separately and labelled properly , 1ml suspension of each isolate was inoculated in each flask according to the labeling. Flasks were kept for incubation for 5 days at room temperature. After incubation 0.5 ml Nessler's reagent was added in each flask and tubes were observed for development of brown to yellow color and results were noted down.

3.11 Antibiotic sensitivity testing

The bacterial isolates 1 ,6 and mixture of 1 and 6 were proceeded for antibiotic sensitivity testing.

Nutrient agar plates were prepared, after solidification of agar medium 0.1 ml suspension of isolates were spread on NA media. In asceptic condition discs of various antibiotics such as, amoxicillin (AMx10), penicillin-G(P10), of loxacin (of5), ciprofloxacin (C15), chloramphenicol (C30), tetracycline (TE30) were placed over NA media plate . Plates were kept for incubation for 24 hrs. at room temperature and observed for inhibitory zone

3.12 Germination studies in petri plates

Germination studies was carried out for isolate 1 , 6 and mixture of 1and 6. Mung beans (*Vigna Radiata)* were taken . Suspension of isolates were prepared .Amount of 10 Moong beans seeds for each were kept for soaking in each suspensions for 10 minutes.

In petri plate, cotton bed was prepared and filter paper was placed over it and was labelled properly, soaked beans were kept over it in appropriate distance. plates were then incubated for 4 days for germination . Germination was observed for every 24 hrs. and length of radical , no. of seed germination was noted down.

3.13 Germination study of tea powder coated seeds

Germination studies of tea powder coated seeds was carried out for isolate 1, 6 and mixture of 1and 6. Mung bean seeds were taken. Suspensions of isolates were prepared and mixed with tea powder in such a way that it easily coats or binds to the mung bean seeds. In petri plate filter paper was placed and labelled properly. Tea powder coated seeds were placed over it and kept for incubation for germination process.

3.14 Germination studies in Jumbo tubes

Germination studies in jumbo tubes was carried out for isolate 1 , 6 and mixture of 1and 6. Suspensions of isolates were prepared and single jowar seed in each were soaked in suspension for 1minute. Jumbo tubes were filled with tea powder and soil in concentration of 9:1 (27gm soil + 3gm tea powder) labeling was done properly and tubes were kept for sterilization. After sterilization the tubes were kept for cooling, treated seeds with suspensions was dumped in soil in jumbo tubes properly according to the labeling in asceptic condition .

The tubes were incubated at room temperature for 5 days . observation was carried out after each 24 hrs. and observed for radicals (shoot length). Length of shoots and leaves were noted down .

Combination of 8 tubes were prepared for comparative study such as ;

Table 1

1	Soil	Seed soaked in distilled water
2	Soil	Seed soaked in suspension of isolate 1
3	Soil	Seed soaked in suspension of isolate 6
4	Soil	Seed soaked in suspension of isolate 1 and 6 mixture
5	Soil + tea powder	Seed soaked in distilled water
6	Soil +tea powder	Seed soaked in suspension of isolate 1
7	Soil + tea powder	Seed soaked in suspension of isolate 6
8	Soil + tea powder	Seed soaked in suspension of isolate 1+6 mixture

3.15 Determination of shelf life of tea powder based bio fertilizer

Tea powder based bio fertilizer was prepared by following ways;

Mixture of tea powder based biofertilizer ;- Ten gm. tea powder was mixed with suspension mixture of 15 ml. of isolate 1 suspension and 15ml. of isolate 6 suspension.

NA media plates were prepared and dilutions of above mixture was carried out in test tubes with proper labeling, 0.1ml. of each dilutions were spread over NA media plates, and plates were labelled properly. All the plates were kept for incubation at room temperature for 24 hrs. and no. of colonies were counted. This procedure was carried out for about interval every week.

CHAPTER 4.0
RESULT AND DISCUSSION

4.0 RESULTS AND DISCUSSION

4.1 Isolation and selection of bacterial isolates

4.1.1 *Collection of soil sample.*

A total of 30 bacterial colonies were isolated from sugarcane soil and banana soil from Kolhapur district in August 2023. Out of 30, 12 isolates were selected for characterization.

4.1.2 *Morphological characteristics and microscopic observation of bacterial isolates .*

The Morphological characteristics of bacterial isolates varied slightly. All the isolates produced round (circular) shaped and white color colonies with convex elevation having smooth surface with undulated entire margin. All 12 isolates showed moist consistency while one of them showed sticky consistency. Whereas no pigmentation was observed on NA media plate. Microscopic observation were performed to investigate the characteristics of bacterial isolates such as shape, Gram nature and motility.

Among 12 , some of them showed short rod , long thick rod and some showed cocci shaped organisms

In gram reaction 9 were Gram positive while remaining isolates were Gram negative in nature.

Fig ; Banana and sugarcane soil

Table 2 MORPHOLOGICAL CHARACTERS

ISOALTES	SIZE	SHAPE	COLOR	OPACITY	MARGIN	ELEVATION	SURFACE	CONSISTENCY
1	1mm	Circular	white	Opaque	Entire	Convex	Smooth	Moist
2	1mm	Circular	White	Opaque	Entire	Convex	Smooth	Moist
3	1mm	Circular	White	Opaque	Entire	Convex	Smooth	Moist
4	1mm	Circular	White	Opaque	Entire	Convex	Smooth	Moist
5	1mm	Circular	White	Opaque	Entire	Convex	Smooth	Moist
6	1mm	Circular	White	Opaque	Entire	Convex	Smooth	Sticky
7	1mm	Circular	White	Opaque	Entire	Convex	Smooth	Moist
8	1mm	Circular	White	Opaque	Entire	Convex	Smooth	Moist
9	1mm	Circular	White	Opaque	Entire	Convex	Smooth	Moist
10	1mm	Circular	White	Opaque	Entire	Convex	Smooth	Moist
11	1mm	Circular	White	Opaque	Entire	Convex	Smooth	Moist
12	1mm	Circular	White	Opaque	Entire	Convex	Smooth	Moist

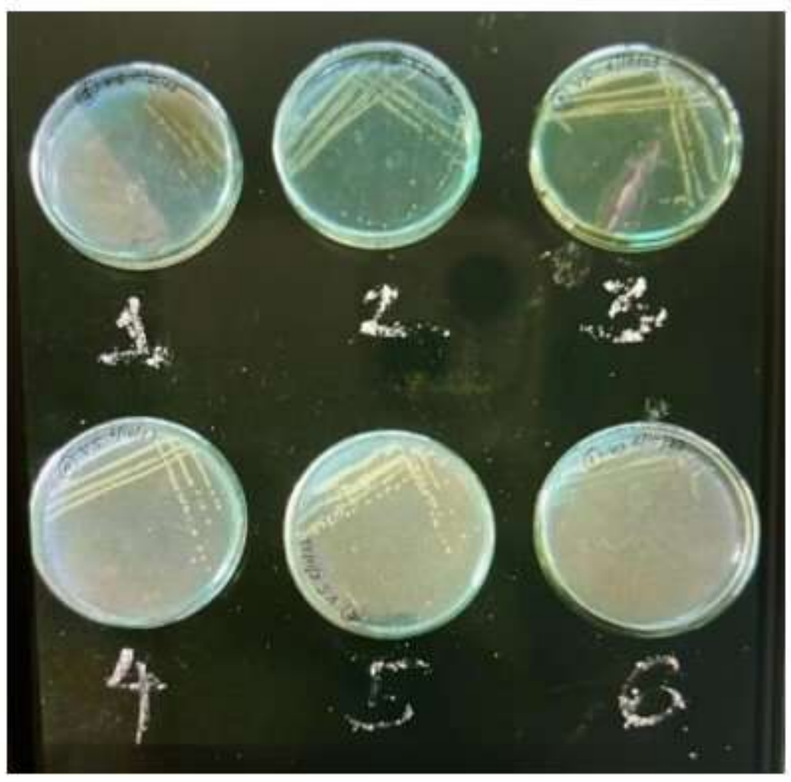


Fig- Growth of isolates

Table 3 MICROSCOPIC OBSERVATIONS

ISOLATE	GRAM STAINING
Isolate 1	Gram positive cocci arranged in cluster`s
Isolate 2	Gram positive short rods arranged singly
Isolate 3	Gram negative short rods arranged singly
Isolate4	Gram positive short rods arranged singly
Isolate5	Gram positive long thick rod arranged in bunch
Isolate6	Gram positive cocci arranged in clusters
Isolate7	Gram positive thick rods arranged in chain
Isolate8	Gram positive short rods arranged singly
Isolate9	Gram positive cocci arranged singly
Isolate10	Gram negative short rods arranged singly
Isolate11	Gram positive thick rods arranged singly
Isolate12	Gram positive short rods arranged singly

4.2 Phosphate solubalization (KB - King's B Agar)

12 of the 8 strains exerted ability for phosphate solubalization on KB medium . Out of 8, 2 strains showed maximum degree of phosphate solubalization. This activity Characterizes that the this bacteria are beneficial bacteria capable of solubilizing organic phosphorus from insoluble compounds. Phosphate solubalization ability of rhizospheric microorganisms is considered to be one of most important traits associated with plant phosphate nutrition.

PSB (Plant solubilizing bacteria) produces Indole 3-acetic acid (IAA) which enables plant cells to grow.

Table 4

ISOLATE	COLONY DIAMETER	ZONE DIAMETER
Isolate 1	0.5cm	1.2cm
Isolate 2	0,4cm	0.6cm
Isolate 3	0.4cm	No zone
Isolate 4	0.5cm	1cm
Isolate 5	0.3cm	No zone
Isolate 6	0.6cm	1.3cm
Isolate 7	0.1cm	0.7cm
Isolate 8	0.3cm	0.5cm
Isolate 9	0.1cm	0.6cm
Isolate 10	0.4cm	No zone
Isolate 11	0.2cm	No zone
Isolate 12	0.2cm	No zone

Fig ; Phospate solubilaztion

4.2 Nitrogen fixation

All of the 12 Isolates showed nitrogen fixation ability on Ashby's Mannitol Agar. Maximum growth of isolate 1 and 6 were observed.

Nitrogen fixation activity characterizes that this bacteria converts nitrogen gas into usable form i.e. ammonia which influences plants growth.

Table 5

ISOLATES	GROWTH
Isolate 1	+
Isolate 2	-
Isolate 3	-
Isolate 4	+
Isolate 5	+
Isolate 6	+
Isolate 7	-
Isolate 8	-
Isolate 9	+
Isolate 10	+
Isolate 11	+
Isolate 12	-

Fig ; Nitrogen fixation

4.3 Identification of potent isolates

On the bases of phosphate solubalization ability and nitrogen fixation ability, two potent isolates were identified i.e. isolate 1 and isolate 6.

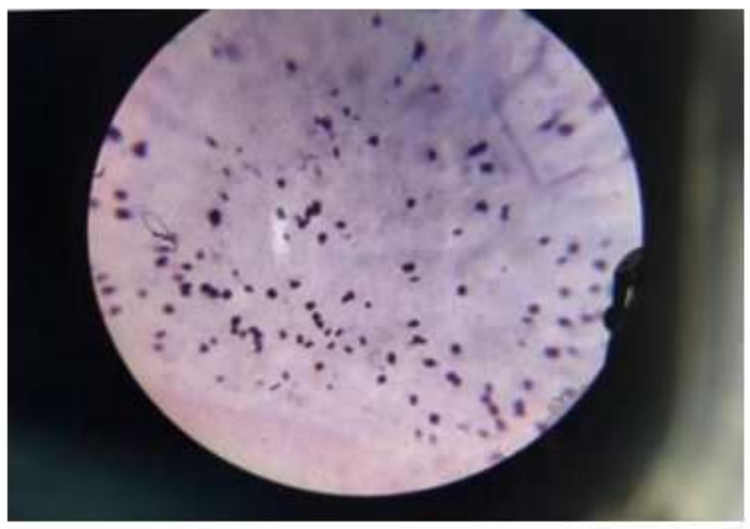
4.3.1 Identification by morphological characters of potent isolates.

Table 6; Morphological characters

Sr.no.	Isolates	size	shape	color	opacity	Margin	elevation	surface	consistency
1	Isolate 1	1mm	circular	White	opaque	Entire	Convex	smooth	Moist
2	Isolate 6	1mm	circular	White	opaque	Entire	Convex	smooth	Sticky

Table 7; Microscopic observation

Sr.no.	Isolates	Gram staining
1	Isolate 1	Gram positive cocci arranged in clusters and singly.
2	Isolate 6	Gram positive cocci arranged in clusters



Fig; Microscopic observation of isolate 1

4.4 NaCl tolerance

Out of 12 , isolate 1 and 6 showed strong NaCl tolerance ability. Turbidity was observed till 7% of NaCl concentration. This indicates that, this isolates have potential to grow and flourish it"s life cycle in high saline conditions.

Table ; 8

ISOLATE	1%	2%	3%	4%	5%	6%	7%	8%	9%	10%
Isolate 1	+++	+++	+++	+++	+++	+++	+++	-	-	-
Isolate 6	+++	+++	+++	+++	+++	+++	+++	-	-	-

Fig ; NaCl tolerance

4.2Potassium chloride effect

According to the NaCl tolerance results , Isolate 1 and 6 were selected to study potassium chloride effect . Maximum turbidity was observed of isolate 1 and 6 till 8% of KCL concentration . This indicates that this isolate are resistant to high KCL concentration.

Table ; 9

ISOLATES	1%	2%	3%	4%	5%	6%	7%	8%	9%	10%
Isolate 1	+++	+++	+++	+++	+++	+++	+++	+++	-	-
Isolate 6	+++	+++	+++	+++	+++	+++	+++	+++	-	-

4.5Sodium carbonate effect

Isolate 1 and 6 were proceeded for a sodium carbonate effect study . The results were negative for this effect as there was no turbidity observed.

Table 10

ISOLATES	0.1%	0.3%	0.5%	0.7%	0.9%	1%
Isolate 1	-	-	-	-	-	-
Isolate 6	-	-	-	-	-	-

4.6 Sodium hydrogen carbonate effect

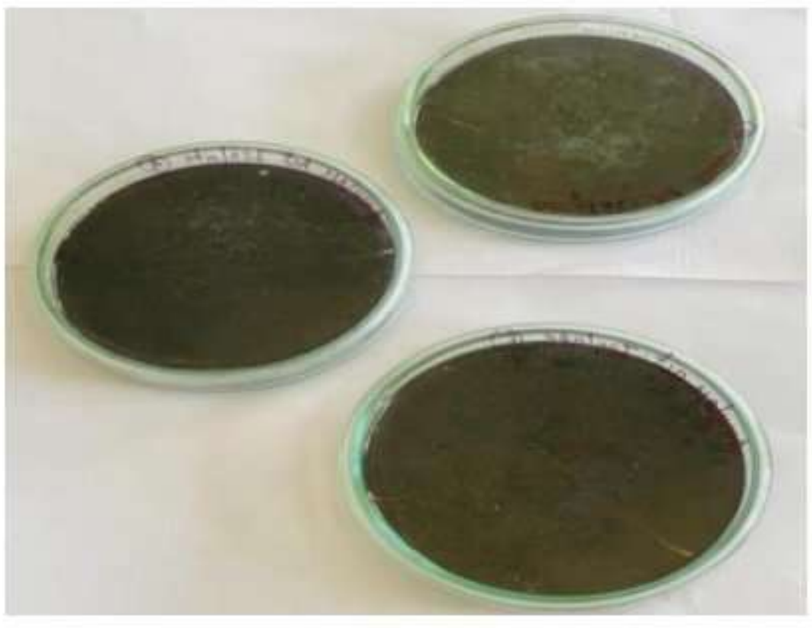
Isolate 1 and 6 were proceeded for the study of sodium hydrogen carbonate effect . The results were negative for this effect as there was no turbidity observed.

Table 11

ISOLATES	0.1%	0.3%	0.5%	0.7%	0.9%	1%
Isolate 1	-	-	-	-	-	-
Isolate 2	-	-	-	-	-	-

4.7 Amylase Activity

Amylase activity was determined on starch agar medium of isolate 1 and 6 .After 24 hrs of incubation plates were flooded with iodine solution for 1 min . Hence the clear zone around colonies was not observed (Fig;) . Therefore test was negative . This indicates that this bacterial isolates cannot hydrolyze starch (amylose and amylopectin) using the enzymes a-amylase.



Fig; Amylase activity

4.8Antibiotic sensitivity

Isolate no. 1 and 6 and mixture of 1 and 6 were proceeded for antibiotic sensitivity testing .

Antibiotic such as Chloramphenicol, Ciprofloxacin, Tetracycline, Penicillin-G , Amoxicillin and Ofloxiacin were used to check the antibiotic sensitivity.

Isolate 1 ,6 and mixture of 1 and 6 showed maximum inhibitory zone against Ciprofloxacin antibiotic . This test measures the ability of a specific organism to grow in the presence of a particular drug in vitro.

Table 12 ISOLATE 1

NAME OF ANTIBIOTICS	SHORT FROMS	INHIBITORY ZONE
Chloramphenicol	C30	1.5
Ciprofloxacin	C15	2
Tetracycline	TE30	1
Penicillin-G	P10	1.7
Amoxicillin	AMX10	1.7
Ofloxiacin	OF5	2

Table 13 ISOLATE 6

NAME OF ANTIBIOTICS	SHORT FROMS	INHIBITORY ZONE
Chloramphenicol	C30	1.5
Ciprofloxacin	C15	2
Tetracycline	TE30	1
Penicillin-G	P10	NO ZONE
Amoxicillin	AMX10	NO ZONE
Ofloxiacin	OF5	2

Table 14 ISOLATE (1+6)

NAME OF ANTIBIOTICS	SHORT FROMS	INHIBITORY ZONE
Chloramphenicol	C30	1.7
Ciprofloxacin	C15	3.25
Tetracycline	TE30	1.2
Penicillin-G	P10	NO ZONE
Amoxicillin	AMX10	NO ZONE
Ofloxiacin	OF5	1.3

4.10 Ammonia production

Ammonia production activity was proceeded for isolate 1 ,6 and mixture of 1 and 6 . The production of Ammonia was observed in all the isolates .

The ammonia is useful for plant directly or indirectly. Ammonia production by the bacteria helps to influence plant growth indirectly

TABLE 15

ISOLATES	24hrs.	48hrs	72hrs.	96hrs
Isolate 1	+	+	+	+
Isolate 2	++	++	++	++
Isolate (1+6)	++	++	++	++

Fig ; Ammonia Production

4.11 Sugar fermentation

Sugar fermentation test was performed for isolate 1 ,6 and mixture of 1 and 6 . Lactose Maltose, Sucrose, Dextrose, Ribose, Galactose, Mannitol, and Glucose solutions were prepared .The test showed positive Ribose and Glucose fermentation and acid formation was observed by color change i.e. formation of pink color .

TABLE 16

SUGAR	ISOLATE 1	ISOLATE 6	ISOLATE (1+6)
Lactose	-	-	-
Maltose	-	-	-
Sucrose	-	-	-
Dextrose	+	+	+
Ribose	+	+	+
Galactose	-	-	-
Mannitol	-	-	-
Glucose	+	+	+

4.12 Germination studies in petri plate

Germination studies in petri plate was carried out for comparative studies of isolate 1 , 6 and mixture of isolate 1 and 6 using Moong beans seeds . Length of radicals were measured and promising results were found . Germination studies results in examining the growth rate of plant .

TABLE ; 17 AFTER 24hrs.

ISOLATE NO.	NO. OF SEEDS SHOWING GERMINATION	LENGTH OF RADICALS	AVERAGE
CONTROL	No germination	-	
1	No germination	-	
6	6	0.6, 0.7, 0.1, 0.2, 0.3, 0.2	0.35
1+6	7	0.6, 0.7, 1.3, 0.1, 0.2 , 1.1 , 0.8	0.68

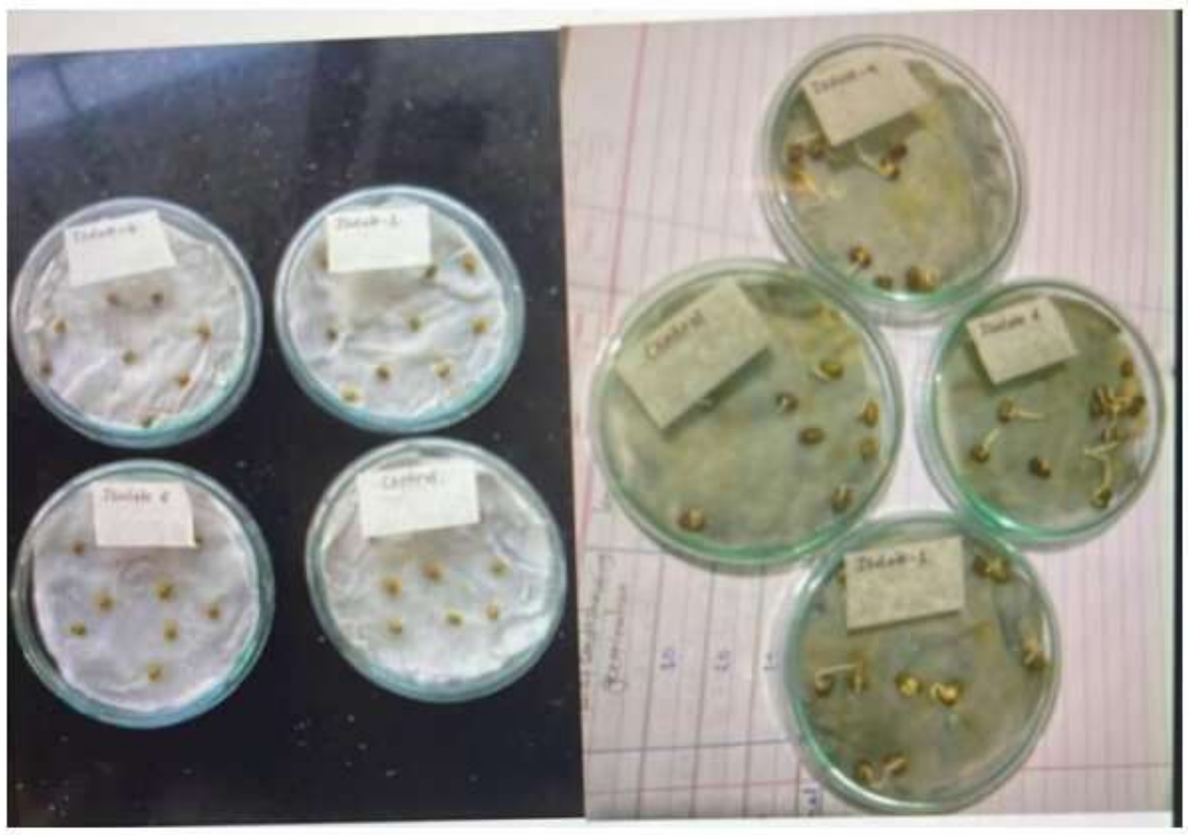


Fig ; Germination after 24 hrs

Table 18 AFTER 48 hrs.

ISOLATE NO.	NO. OF SEEDS SHOWING GERMINATION	LENGTH OF RADICALS	AVERAGE
CONTROL	10	0.1, 0.2, 0.3, 0.7, 1, 0.6, 0.1, 0.3, 0.6, 0.4	0.43
1	10	0.1, 0.2, 0.4, 0.7, 0.3, 1, 0.8, 0.2, 0.6, 1.2	0.47
6	10	0.6, 0.7, 0.1, 0.2, 0.3, 0.2, 0.4, 1.2, 1.3, 0.7	0.57
1+6	10	0.6, 0.7, 1.3, 0.1, 0.2 , 1.1 , 0.8, 1.4, 0.9, 1.5	0.86

Fig ; Germination after 48 hrs

Table 19 AFTER 72 hrs.

ISOLATE NO.	NO. OF SEEDS SHOWING GERMINATION	LENGTH OF RADICALS	AVERAGE
CONTROL	10	0.1, 0.2, 0.4, 0.8, 1.1, 0.7, 0.2, 0.4, 0.7, 0.5	0.51
1	10	1.2, 1.3, 1.6, 3.8, 1.4, 1.2, 1.9, 1.3, 2.8, 2.4	1.89
6	10	1.8, 3.8, 2.3, 4.4, 2.5, 3.4, 1.8, 2.5, 1.7, 2.6	2.68
1+6	10	2.9, 2.8, 2.4, 4.5, 3.2 , 3 , 3.9, 1.6, 2.8, 2	2.91

Fig ; Germination after 72 hrs

Table 20 AFTER 96hrs.

ISOLATE NO.	NO. OF SEEDS SHOWING GERMINATION	LENGTH OF RADICALS	AVERAGE
CONTROL	10	0.9, 0.7, 0.9, 1.8, 1.6, 2.7, 3.2, 4.4, 2.7, 3.5	2.24
1	10	2.2, 2.3, 2.6, 3.8, 2.4, 3.2, 3.9, 3.3, 3.8, 4.4	3.19
6	10	2.8, 4.8, 3.3, 3.4, 3.5, 4.4, 4.8, 5.5, 5.7, 4.6	4.28
(1+6)	10	4.2, , 4.4, 4, 2.9 , 3.4 , 4.4, 5.6, 6.8, 7, 8	5.07

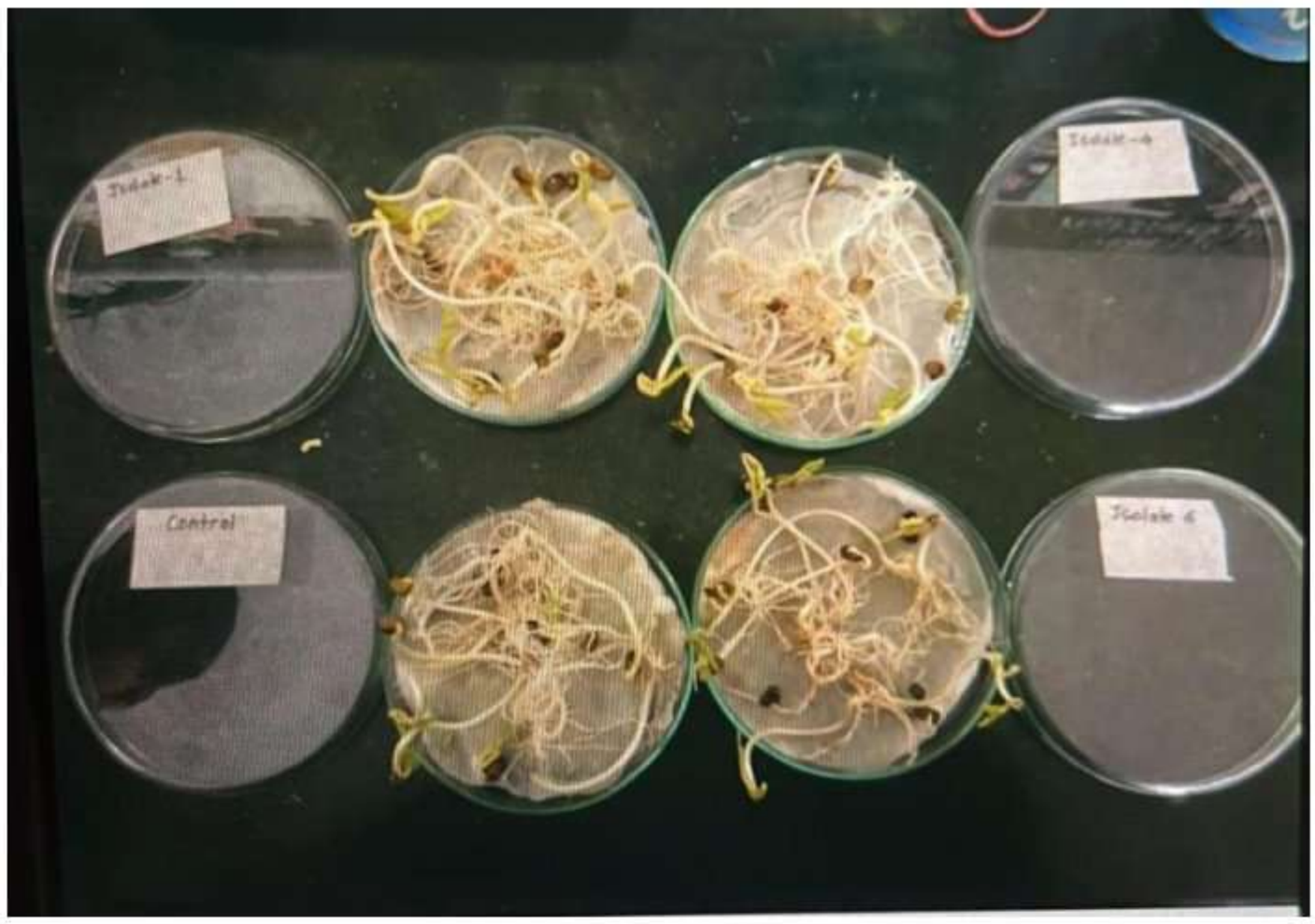


Fig ; Germination after 96 hrs

4.13 Germination study of tea powder coated seeds.

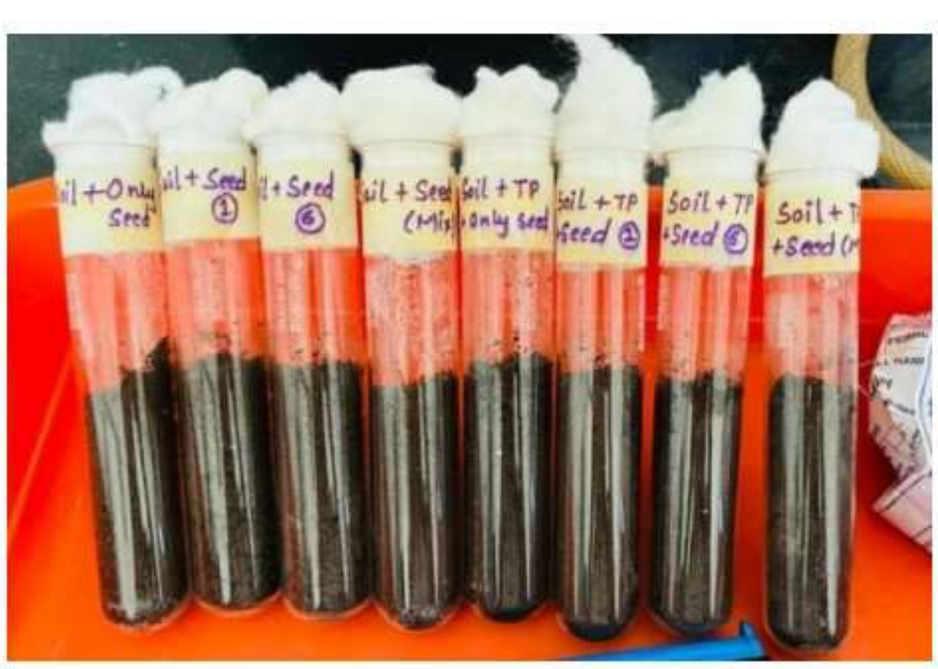
Germination studies of tea powder coated seeds were carried out to observe the efficiency of growth with isolates and seeds.

Fig ; Germination after 24 hrs

Fig ; Germination after 48 hrs

4.14 Germination studies in jumbo tubes

Germination studies in jumbo tubes was carried out for comparative studies of isolate 1 , 6 and mixture of isolate 1 and 6 using jowar seeds and mixture of soil and tea powder . length of radicals were measured.



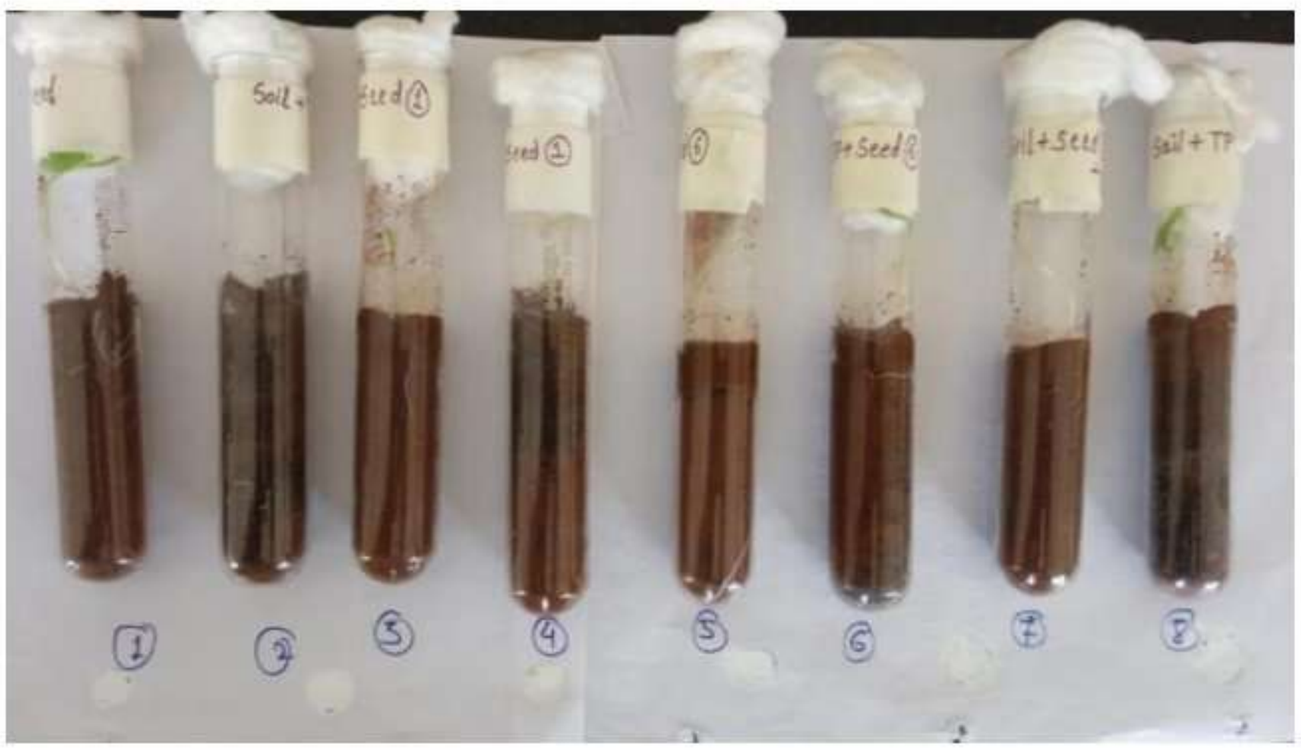


Fig ; Germination in jumbo tubes

TABLE 21

SR.NO	COMBINATIONS	ROOT LENGTH (cm)	SHOOT LENGTH (cm)	LEAF LENGTH (cm)
1	Soil + seed	7.3	4	1.5
2	Soil + seed + isolate 1	5.5	5.8	1.5
3	Soil + seed + isolate 6	7.5	6	1.5, 2
4	Soil+ seed + isolate (1+6)	8.9	5.7	2.7, 0.5
5	Soil +tea powder + seed	-	-	-
6	Soil +tea powder +isolate 1	5.9	5.8	1.5
7	Soil + tea powder + isolate 6	9.3	5	2.7
8	Soil + tea powder + isolate (1+6)	10	6.7	2.5 , 1

4.15 Shelf life

Shelf life of tea based bio fertilizer was carried out by SPC method . It is necessary to determine the life of microorganism to stay with carrier for long period. Promising results were obtained.

Table 22 After 1 week

Sr.no.	Dilutions	No of colonies
1	1:10	Uncountable
2	1:1000	Uncountable
3	1:100000	Uncountable
4	1:10000000	98
5	1:1000000000	25

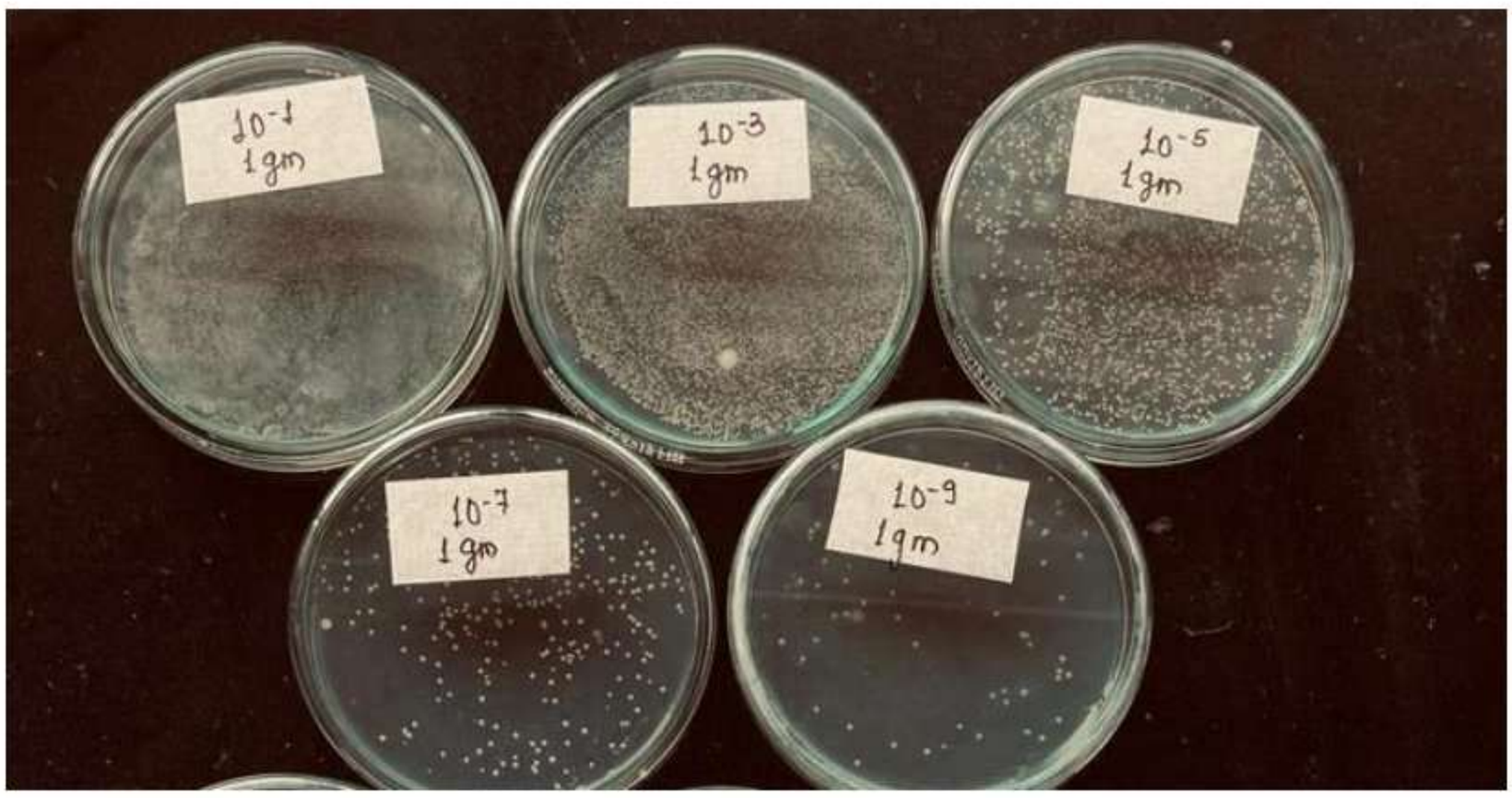


Fig ; After one week

Table 23 after 2 weeks

Sr.no.	Dilutions	No. of colonies
1	1:10	Uncountable
2	1:1000	Uncountable
3	1:100000	Uncountable
4	1:10000000	Uncountable
5	1:1000000000	Uncountable

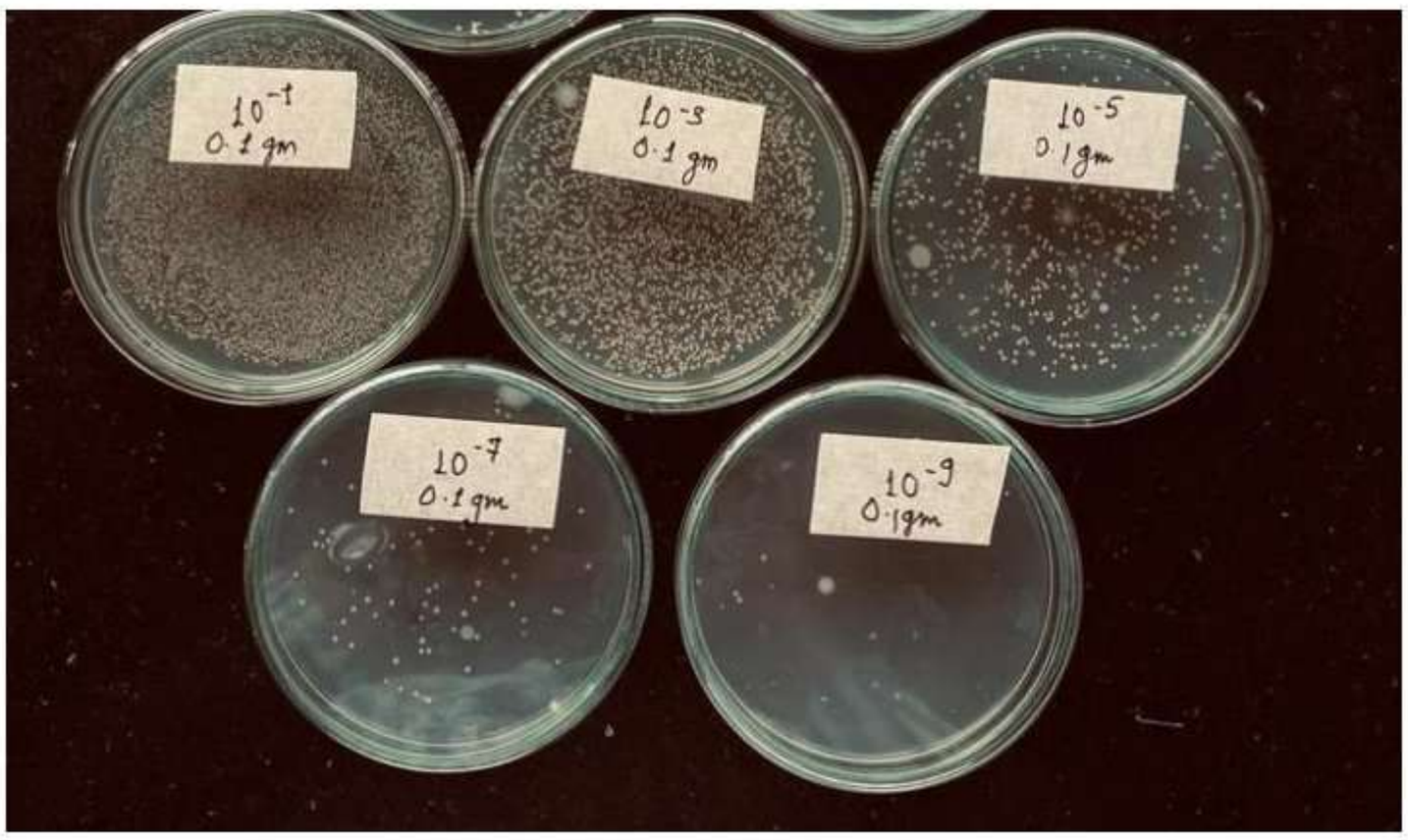


Fig ; After two weeks

CHAPTER 5.0
SUMMARY AND CONCLUSION

5.0Summary and conclusion

Soil samples were collected from banana field of Sangli district and sugarcane field of Kolhapur district and was brought to laboratory immediately.

Soil was then examined by morphological test, Gram staining and biochemical tests, for the isolation of potent isolates .

Potent isolates was then tested by KB agar medium for phosphate solubalization ability, this activity characterizes that theses bacterial isolates are capable of solubilizing organic phosphorous from insoluble compounds. It is one of the most important traits associated with plant phosphate nutrition.

Nitrogen fixation ability was carried out of potent isolates to check the conversion of nitrogen gas into usable form of ammonia which influences plant growth.

NaCl tolerance, potassium chloride effect, sodium carbonate effect, sodium hydrogen carbonate effect was carried out to determine the potential of potent isolates to grow and flourish its life cycle in high salt condition.

Amylase activity was carried out using starch agar medium to determine the hydroxylation of starch using the enzyme a-amylase.

Antibiotic sensitivity was carried out using various antibiotics for potent isolates and promising results were determined as the isolates were sensitive to the antibiotics, this results in growth of plant in presence of drug as well.

Ammonia production was carried out to examine production of ammonia by potent isolates which helps to influence plant growth indirectly.

Sugar fermentation showed positive results for dextrose and ribose sugar which produced acids.

Germination studies in petri plates, germination studies by tea powder coated seeds and germination studies in jumbo tubes were carried out for comparative studies and to determine the growth rate of shoot length , leaf length and root length.

As there is a growing demand for organic foods in global market ,shelf life of bio inoculant plays important role in agriculture field . Charcoal based biofertlizer last for 6 months and whereas tea powder based biofertlizer last for more than 6 months .

As we know peat, wood charcoal and lignite based bio fertilizers suffers from poor quality, high contamination and unpredictable field performance , following study of formulation of carrier based bacterial inoculant using waste tea powder was successfully carried out by exerting promising results. The tea powder based bacterial inoculant showed high germination studies which can results in increase in agriculture yields and offer framer an economical natural fertilizer. As our produced bio inoculant is solid form of biofertlizer it helps to promote plant growth by improving nutrient acquisition

CHAPTER 6.0
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6.0Bibliography

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