# PREPARATION OF CULTURE MEDIA FROM THE PEEL OF FRUITS AND VEGETABLES

*Submitted to Bharathiar University*

*For the Award of the Degree of*

**MASTER OF SCIENCE**

**IN**

**APPLIED MICROBIOLOGY**

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**Reaccredited at A Level with cGPA (3.53/4)**

**COIMBATORE, TN, INDIA**

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## DECLARATION

I **JABIYA ELIZA VARUGHESE** hereby declare that the thesis entitled "**PREPARATION OF CULTURE MEDIA FROM FRUIT AND VEGETABLE PEEL**" submitted to the Bharathiar University, in partial fulfilment of the requirements for the award of the Degree of Master of Science in Microbiology is a record of original and independent research work done by me during February 2021 under the supervision and guidance of **Mr. B. Venkatrajah**, Assistant Professor of Microbiology, Department of Microbiology, School of Biological Sciences, CMS College of Science and Commerce, Coimbatore, and it has not formed the basis for the award of any Degree/ Diploma/ Associate ship/ Fellowship or other similar title to any candidate in any University.

**JABIYA ELIZA VARUGHESE**

## CERTIFICATE

This is to certify that the thesis entitled "**PREPARATION OF CULTURE MEDIA FROM FRUIT AND VEGETABLE PEEL**" submitted to the Bharathiar University, in partial fulfilment of the requirements for the award of the Degree of Master of Science in Microbiology is a record of original research work done by **JABIYA ELIZA VARUGHESE**, during the period of her study in the Department of Microbiology, School of Biological Sciences, CMS College of Science and Commerce, Coimbatore, under my supervision and guidance and the thesis has not formed the basis for the award of any Degree/ Diploma/ Associate ship/ Fellowship or other similar title to any candidate in any University.

Counter Signed

Signature of the Guide

DIRECTOR

Mr. B. Venkatrajah

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**JABIYA ELIZA VARUGHESE**

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**CONTENTS**

# ABSTRACT

## ABSTRACT

In this project culture media is prepared from waste peel of fruits and vegetables like orange, lemon and carrot respectively. The peels are dried and made into powdered form. These powders are subjected to trial test to identify the best concentration of the sample for better growth of the fungus and bacteria. The appropriate concentration is taken and made into media using agar as the solidifying agent. The organisms such bacteria and fungus are isolated from the soil and test in the new media by streak inoculation and waits for 24hr for the formation of growth in it.

The results will be noted and recorded if growth is observed or not.

# INTRODUCTION

# 1. INTRODUCTION

Fruit and vegetable peels can reduce hunger and help you feel fuller for longer. This is largely due to their high fibre content. While the exact amount of fibre varies, fresh fruits and vegetables may contain up to one-third more fibre before the outer layers are removed. Fibre also serves as food for the friendly bacteria living in your gut. When these bacteria feed on fibre, they produce short-chain fatty acids. While both fruits and vegetables are a great source of nutrients, essential for overall health, their skins and peels are equally important. Although the peels may not look appetising, it can be extremely beneficial in boosting your metabolism, controlling blood pressure and improving eyesight, to a name few. From orange and pomegranate to potato and onion have a mesmerizing nutrient quantity, for example,

**Orange**: Orange peels are a great option to get rid of pimples, blackheads, dead cells, blemishes and dark circles. It also helps dry skin stay hydrated and adds glow to your face. You can mix water and orange peel (dried and grounded) to your scalp as it helps get rid of dandruff. It is also good for reducing digestion problems.

**Potatoes**: While most of us throw away potato peels, very few know that it is filled with nutrients like iron, calcium, potassium, vitamins and magnesium. It helps boost metabolism and helps control blood sugar. Being an antibacterial agent, potato skin is good for human skin. Besides, it also prevents cancer.

## 1.1. CULTURE MEDIA

Culture media contains nutrients and physical growth parameters necessary for microbial growth. All microorganisms cannot grow in a single culture medium and in fact many can't grow in any known culture media.

Organisms that cannot grow in artificial culture medium are known as obligate parasites. *Mycobacterium leprae*, *Rickettsias, Chlamydias,* and *Treponema palladium* are obligate parasites. Bacterial cultures media can be distinguished on the basis of composition, consistency and purpose.

### 1.1.1. Classification of Culture Media Used in Microbiology Laboratory on the basis of Consistency

1. **Solid Medium**

   Solid medium contains agar at a concentration of 1.5-2.0% or some other, mostly inert solidifying agent. Solid medium has physical structure and allows bacteria to grow in physically informative or useful ways. Solid medium is useful for isolating bacteria or for determining the colony characteristics of the isolate.

2. **Semi Solid Media**

   They are prepared with agar at concentrations of 0.5% or less. They have soft custard like consistency and are useful for the cultivation of microaerophilic bacteria or for determination of bacterial motility.

**3. Liquid Medium**

These media contains specific amounts of nutrients but don't have trace of gelling agents such as gelatine or agar, broth medium serves various purposes such as propagation of large number of organisms, fermentations studies, and various other tests. Example, sugar fermentation test, MR VP broth.

### 1.1.2 Classification of Culture Media Based on the Basis of Composition

1. Synthetic or Chemically Defined Medium

A chemically defined medium is one prepared from purified ingredients and therefore whose extract composition is known.

2. Non Synthetic or Chemically Undefined Medium

Non synthetic medium contains at least one component that is neither purified nor completely characterised nor even completely consistent from batch to batch. Often these are partially digested proteins from various organism sources. Nutrient broth, for example, is derived from cultures of yeasts.

Synthetic medium maybe simple or complex depending upon the supplement incorporated in it. A simple non synthetic medium is capable of meeting the nutrient requirements of organisms requiring relatively few growth factors whereas complex non synthetic medium support the growth of more fastidious microorganisms.

### 1.1.2. Classification of Bacterial Culture Media based on the basis of Purpose/ Functional use/ Application

Many special purpose media are needed to facilitate recognition, enumeration, and isolation of certain types of bacteria. To meet these needs numerous media are available.

**1. General Purpose Media/ Basic Media**

Basal media are basically simple media that supports most non fastidious bacteria. Peptone water, nutrient broth and nutrient agar are considered as basal media. These media are generally used for the primary isolation of microorganisms.

**2. Enriched media (Added growth factors)**

**Blood Agar**

Addition of extra nutrients in form of blood, serum, egg yolk etc. to basal medium makes them enriched media. Enriched are used to grow nutritionally exacting (fastidious) bacteria. Blood agar, Chocolate agar, Loffler's serum slope etc. are few of the enriched

media. Blood agar is prepared by adding 5-10% (by volume) blood to a blood agar base. Chocolate agar is also known as heated blood agar or lysed blood agar.

**3. Selective and Enriched Media**

These are designed to inhibit unwanted commensal or contaminating bacteria and help to recover pathogen from a mixture of bacteria. While selective media are agar based, enrichment media are liquid in consistency. Both these media serve the same purpose. Any agar media can be made selective by addition of certain inhibitory agents that don't affect the pathogen of interest. Various approaches to make a medium selective include addition of antibiotics, dyes, chemicals, alteration of pH or a combination of these.

**a. Selective medium**

**Principle:** Differential growth suppression selective medium is designed to suppress the growth of some microorganisms while allowing the growth of others. Selective medium are agar based (solid) medium so that individual colonies may be isolated.

**Examples of selective media include:**

1. Thayer Martin Agar used to recover *Neisseria gonorrhoea* contains antibiotics; vancomycin, colistin and nystatin.

2. Mannitol salt agar and Salt milk agar used to recover *S. Aureus* contains 10% NaCl.

3. Potassium tellurite medium used to recover *C. diphtheriae* contains 0.04% potassium tellurite.

4. Mac Conkey's agar used for *Enterobacteriaceae* members contains bile salt that inhibits most gram positive bacteria.

5. Crystal violet Blood Agar used to recover *S.pyogenes* contains 0.002% crystal violet.

6. Lowenstein Jensen Medium used to recover *M.tuberculosis* is made selective by incorporating malachite green.

7. Wilson and Blair's Agar for recovering *S.typhi* is rendered selective by the addition of dye brilliant green.

8. Selective media such as TCBS Agar used for isolating *V.cholerae* from faecal specimens have elevated pH (8.5-8.6), which inhibits most other bacteria.

**b. Enrichment Culture Medium**

Enrichment culture media is used to increase the relative concentration of certain microorganisms in the culture prior to platting on solid selective medium. Unlike selective media, enrichment culture is typically used as broth medium.

Enrichment media are liquid media that also serves to inhibit commensals in the clinical specimen. Selenite F broth, tetrathionate broth and alkaline peptone water (APW) are used to recover pathogens from faecal specimens.

**4. DIFFERENTIAL / INDICATOR MEDIUM:**

Certain media are designed in such a way that different bacteria can be recognized on the basis of their colony colour. Various approaches include incorporation of dyes, metabolic substances etc. so that those bacteria that utilize them appear as differently coloured colonies. Such media are called differential media or indicator media. Differential media allow the growth of more than one microorganism of interest but with morphologically distinguishable colonies.

**Examples of differential media include:**

1. Mannitol salts agar (Mannitol fermentation = yellow)

2. Blood agar (various kinds of haemolysis)

3. Mac Conkey agar (lactose fermenters, pink colonies where as non-lactose fermenter produces pale or colourless colonies.

4. TCBS (*Vibrio cholerae* produces yellow colonies due to fermentation of Sucrose)

**5. TRANSPORT MEDIA:**

Clinical Specimens must be transported to the laboratory immediately after collection to prevent overgrowth of contaminating organisms or commensals. This can be achieved by using transport media. Such media prevent drying of specimen, maintain the pathogen to commensal ratio and inhibit overgrowth of unwanted bacteria. Some of these media are;

- Cary Blair transport medium us Venkataramen Ramakrishnan (VR) *Streptococci* from throat specimen

## 6. ANAEROBIC MEDIA

Anaerobic bacteria need special media for growth because they need low oxygen content, reduced oxidation-reduction potential and extra nutrients.

- Robert Cooked Meat (RCM) medium that is commonly used to grow Clostridium species contains a 2.5 cm column of heat meat and 15 ml of nutrient broth. Methylene blue or resazurin is an oxidation potential indicator that is incorporated in the medium. Under reduced condition, methylene blue is colourless.

## 7. ASSAY MEDIA

These media are used for the assay of vitamins, amino acids and antibiotics. For example, antibiotic assay media are used for determining antibiotic potency by the microbiological assay technique.

Other type of medium includes;

- Media for enumeration of bacteria
- Media for characterization of bacteria
- Maintenance media etc...

# AIM AND OBJECTIVE

**AIM:**

To prepare culture media for different bacteria and fungus from fruit and vegetable peel.

**OBJECTIVE:**

1. To prepare culture media for different bacteria from the peels of fruit and vegetable.
2. To prepare culture media for different fungus from the peels of fruit and vegetable

# REVIEW OF LITERATURE

## REVIEW OF LITERATURE

Preparation of culture media from the slime extracted from fresh water snail. (Jabiya Eliza Varughese, Ajmiya S, Naufia N)

Concentrated starter cultures are now used routinely in many food fermentations. Recent literature concerning preparations and performance of such cultures is reviewed. (Journal of Milk and Food Technology.SE Gilliland, ML Speck)

While the literature documents the universal occurrence of heterotrophic plate count (HPC) bacteria in soils, foods, air, and all sources of water, there is a lingering question as to whether this group of organism may signal an increased health risk when elevated populations are present in drinking water (Martin J Allen, Stephen C Edberg, Donald J Reasoner)

The methods of culture were discussed for the isolation and routine cultivation of a group of thermophilic anaerobic spore-forming bacteria. It seemed necessary to study the technique of culture of this particular group in some detail as in many instances it was found that either media or methods which have been used by other investigators for the various groups of mesophlic anaerobes were not satisfactory for the routine study of this group. (Journal of bacteriology 29, LS McClung)

The growth responses of *Salmonellae* as affected by NaCl concentration, pH level and storage temperature were studied in laboratory medium. Growth curves were obtained at 5 concentrations of NaCl, 5pH levels and 5 storage temperatures. (International Journal of Food Microbiology. Angela M Gibson, N Bratchell)

It had long been assumed that a bacteria cell was dead when it was no longer able to grow on routine culture media. We now know that this assumption is simplistic, and that there are many situations where a cell loses culturability but remains viable and potentially able to regrow. (The Journal of Microbiology43, James D Oliver)

Anaerobic bacteria were found in mixed culture specimens from four of ten tested cases of chronic secretory otitis media. These anaerobic bacteria were in a mixed infection flora with aerobic bacteria, most often *Staphylococcus epidermidis* and *Corynebacterium* sp. (Annals of Otology, Rhinology & Laryngology 86, and RS Fulghum)

An improved rumen fluid agar medium was developed that permitted the growth of about double the numbers of bacteria from ruminal contents grown in the medium previously used. (Journal of Diary Science 44, M Pu Bryant)

Elicitation of induced systemic resistance (ISR) by plant-associated bacteria was initially demonstrated using *Pseudomonas* spp. and other gram-negative bacteria. Fewer published accounts of ISR by *Bacillus* spp. are available. (Phytopathology 94, Joseph W Kloepper)

Incorporation of 1.9% β-disodium glycerophosphate (GP) into a complex medium resulted in improved growth by lactic *Streptococci* at 30 C. The medium, called M17, contained: Phytone peptone, 5.0g; polypeptone, 5.0g; yeast extract, 2.5 g; beef extract, 5.0 g; ascorbic acid, 0.5 g; GP, 19.0g; 1.0 M MgSO4.7H20,1.0 ml; and glass-distilled water ,1,000 ml.(Applications of Environmental Microbiology 29, Betty E Terzaghi)

The preservation of microorganisms by different drying methodologies has been use for decades. Freeze drying in particular is the preferred method for transporting and storing vast culture collections of microorganisms strain types. (Journal of Microbiological methods 66, Charlote A Morgan)

With knowledge of the basic concepts of medium composition and the physical conditions which may limit microbial growth, one can enhance the ability to grow bacteria and fungi in pure culture and to enrich for, isolate, and culture many microorganisms of interest from the environment. (Manual of Environmental Microbiology, Ralph S Tanner)

Green macroalgae, such as Ulvales, lose their typical morphology completely when gown under axenic conditions or in the absence of the appropriate micro biome. (Frontiers in Plant Science6, Thomas Wichard)

The overall growth kinetics of four potentially probiotic strains (*Lactobacillus fermentum)* cultured in malt, barley and wheat media were investigated. His objectives were to identify the main factors influencing the growth and metabolic activity of each strain in association with the cereal substrate. (Journal of Applied Microbiology92, D Charalampopoulos et al., 2002)

Few developments in microbiological diagnostics have had such a rapid impact on species level identification of microorganisms as matrix-assisted laser desorption/ionization time of flight mass spectrometry. Conventional differentiation methods rely on biochemical criteria and require additional pre-testing and lengthy incubation procedures. (Applied Microbiology and Biotechnology93, Andreas Wieser et al.)

Ideally, invading bacteria are detected as early as possible in critically ill patients: the strain of morbific pathogens is identified rapidly and antimicrobial therapy. (PLoS pathogens 9(5), Lieuwe DJ Bos et al., 2013)

The use of colloidal clay as a substrate for growing plants suggested itself as a transition step from the aqueous to the soil culture methods. Since speed of chemical activity increases as the total surface of a constant mass increases, it is logical to attempt the analysis of the chemical interrelations of the soil and the plant roots by studying them first by using only the clay, or the most active separate of the soil.(Soil Science 62, Wma Albercht et al.,1946)

Difficulties with filtration in the preparation of egg yolk media were overcome by preparing the yolk emulsion with distilled water instead of saline. The turbidity of the final medium was related to its sodium chloride content. (Journal of Applied Bacteriology 20, EVE Billing et al., 1957)

The establishment of human term trophoblast cells in culture is dependent on the method of cell preparation, growth medium used, and presence of serum. (The journal of Clinical Endocrinology & Metabolism69, Juliet O Lobo, Francis L Bellino)

Platelet derived growth factors have been shown to stimulate cell proliferation efficiently in vivo 1, 2 and in vitro. This effect has been reported for mesenchymal stromal cells, fibroblasts and endothelial colony forming cells with platelets activated by thrombin 3-5 or lysed by freeze/thaw cycles 6-14 before the platelet releasate is added to the cell culture medium.(Journal of visualized experiments: JoVE, 2009 )

Plaque assays in cell culture monolayers under solid or semisolid overlay media are commonly used for quantification of viruses and antiviral substances. To overcome the pitfalls of known overlays, we tested suspensions of microcrystalline cellulose Avicel RC/CL as overlay media in the plaque-inhibition assay of influenza viruses. (Virology journal 3(1), Mikhail Matrosovich, Tatyana Matrosovich, Wolfgang Garten, Hans-Dieter Klenk)

A device for selective plane illumination microscopy (SPIM) of three-dimensional multicellular spheroids, in culture medium under stationary or micro fluidic conditions, is described. (Journal of biomedical optics 17(10) 2012, Thomas Bruns, Sarah Schickinger, Rainer Witting, Herbert Schneckenburger)

Rubella hemagglutinin prepared in serum free suspension culture of BHK-21 cells. The authors have developed an improved method for preparing rubella virus. (Ann. Med Exper. et biol. Fenniae 45(2), 1967, PE Halonen, JA Stewart, AD Hall)

The medium, the full composition of which is given, is a modification of the semi-defined medium previously described. The 10% fetal calf serum has been replaced with a purine base, vitamin, albumin, mixture based on the defined media REI and HX25. (Journal of Parasitology 64(1), Randolph L Berens, JJ MARK)

The preparation and use of a simple culture medium for *Leptospirae*. The description of a new and simple culture medium for *Leptospira.* (Journal of Pathology and Bacteriology 58 (3), 1946, RD Stuart)

# METERIALS AND METHODS

# MATERIALS AND METHODS

## MATERIALS:

1. Sample
    - Orange peel powder
    - Lemon peel powder
    - Carrot peel powder
2. Media
    - Nutrient agar
    - SDA
    - Potato dextrose agar
    - Rose Bengal agar
    - Nutrient broth
    - Biochemical medias
3. Agar agar
4. Glass wares
    - Conical flasks
    - Beakers
    - Pertri plates
    - Test tubes
    - Measuring cylinder
    - Glass slides
    - Pipettes
5. Inoculum loop
6. Distilled water
7. Bunsen burner
8. Dyes
    - Crystal violet
    - Grams iodine
    - Decolouriser
    - Safranin

**INSTRUMENTS**

- Hot air over
- Auto clave
- Incubator
- LAF
- Water bath
- Microwave oven

**METHODS:**

**PROCEDURE:**

**SAMPLE COLLECTION:**

Oranges, lemon and carrot were bought from the market and washed properly under running tap water. The peels were removed from the fruit and vegetable respectively. Peels were then dried under shade for almost a month, later it was been made in powder form using a mixer. The powders then were packed in different clean container with lid shown in fig.1.

**ISOLATION OF PURE CULTURES (Fungus and Bacteria)**

Soil sample were collected from different areas of college and were serially diluted in sterile distilled water. Nutrient agar and SDA were prepared, made sterile and poured in sterile petri plate and allowed to set. Using sterile pipette $10^{-2}$ and $10^{-3}$ aliquots from serial dilution were taken and spread plate technique is employed in which 0.1ml of sample is carefully using a sterile pipette is transferred into a sterile culture media (NA and SDA) and spread evenly using L-rod where the plate was kept over the rotating table. After inoculation the plates were kept for incubation in an incubator at 37°C for bacteria foe 24hs and room temperature for fungus for 3-5 days.

After incubation the plates were observed and from the mixed colonies bacteria and fungus were stained using the technique of Gram staining and LPCB respectively. Then after identifying the organism, they were made pure culture and then set for biochemical tests for further identification. The bacteria and fungus were maintained in pure culture form in broth and plates for the project.

**TRIAL TEST**

**PROCEDURE**

- Concentration of 1%, 2% and 3% media broth of orange peel, lemon peel and carrot peel is prepared in tubes (in set of 2) and 1 set is kept for sterilization in autoclave and other set in microwave at low temperature.
- The 2 sets of tube were inoculated with bacteria and fungus and kept for incubation.

- The 2 sets of 3 different media containing bacteria and fungus after incubation were observed and recoded.

**PREPARATION OF CULTURE MEDIA FOR FUNGUS**

**PROCEDURE**

- 100ml of orange, lemon and carrot media (2gm each for 100 ml) is prepared, 2- 2.5gm of agar is added to it (lemon media require 5% agar in it) and the media is set to sterilization in autoclave.
- After sterilization the medias were taken out and poured in sterile petri plates.
- As soon as the plates were set, from fungal pure culture, *Aspergillus, Pencillium, Fusarium* and *Mucor* were inoculated in it.
- The plates were then kept for incubation at room temperature for 3-5 days.
- After incubation the plates were observed and results were noted down.

**PREPARATION OF CULTURE MEDIA FOR BATERIA**

**PROCEDURE**

- 200ml of orange, lemon and carrot media (2gm each for 100 ml) is prepared, 4- 4.5gm of agar is added to it (lemon media require 5% agar in it) and the media is set to sterilization in autoclave.
- After sterilization the medias were taken out and poured in sterile petri plates.
  As soon as the plates were set, from bacterial pure culture, *E-coli, Staphylococcus aureus, Pseudomonas, Bacillus, Streptococcus, Klebsiella* and *Shigella* were inoculated in it.
- The plates were then kept for incubation in an incubator at 37°C for 24hr.
- The plates were observed for growth and the results were recorded.

# RESULT

## RESULTS

### ISOLATION OF PURE CULTURE (Fungus and Bacteria)

After incubation the plates showed mixed colonies of bacteria and fungus in clumped condition in separate plates respectively. There were total of 7 bacterial cultures (marked as S1-S7) and 4 fungal cultures (marked as F1-F4) obtained by pour plate technique and those cultures were then subjected to pure culture form in separate plates respectively.

### GRAM STAINING

Gram staining showed gram positive cocci and rods as well as gram negative rods.

### BIOCHEMICAL TEST

| **TESTS** | **S1** | **S2** | **S3** | **S4** | **S5** | **S6** | **S7** |
|---|---|---|---|---|---|---|---|
| Gram staining | Negative rods | Positive cocci | Negative rods | Positive bacilli | Positive cocci | Negative rods | Negative rods |
| Catalase test | Positive | Positive | Positive | Positive | Negative | Positive | Positive |
| Oxidase test | Negative | Negative | Positive | Negative | Negative | Negative | Negative |
| Indole test | Positive | Negative | Negative | Negative | Negative | Negative | Positive |
| MR test | Positive | Positive | Negative | Negative | Positive | Negative | Positive |
| VP test | Negative | Positive | Negative | Negative | Negative | Positive | Negative |
| Citrate test | Negative | Negative | Positive | Negative | Negative | Positive | Negative |
| Urease test | Negative | Positive | Negative | Positive | Negative | Positive | Negative |
| TSI test | A\A | A\A | AK\AK | A\A | A\A | A\A | A\AK |
| Hanging drop | Motile | Non-motile | Motile | Motile | Non-motile | Non-motile | Non-motile |

## IDENTIFICATION OF BATERIA

**S1 –** *E.coli*

**S2 –** *Staphylococcus*

**S3 –** *Pseudomonas*

**S4 –** *Bacillus*

**S5 –** *Streptococcus*

**S6 –** *Klebsiella*

**S7 –** *Shigella*

## MACROSCOPIC FEATURES OF FUNGAL PLATES

**F1** – Surface is covered with green colour conidia and is granular. Reverse is pale yellow or ivory.

**F2** – Colonies are characteristically blue green with a white periphery and a white reverse topography is rugose.

**F3** – Initially colonies are white and velvety. Later it becomes woolly to cottony. Surface is yellow or buff pink.

**F4** – Cottony to woolly grey colonies that rapidly fill the petridish or test tubes. The surface is covered with dark spores when sporangia develop.

**LACTO PHENOL COTTON BLUE**

**F1** – Hyphae are hyaline, conidiophores are white and terminate in a vesicle. Vesicles are spherical ranging from 45-75µm in diameter and bear phialide which contain chain of conidia which are arranged in basipetally.

**F2** – Hyphae are relatively thin, hyaline and septate. The conidiophores are erect, septate and hyaline to lightly colour. The phialide are flask shaped with blunt ends; usually they are aggregate in whorls at the tip of the metula or directly on a branch of the conidiophore. The ovoid to elliptical conidia are small, unicellular and hyaline or lightly pigmented; the walls maybe rough.

**F3** – Hyphae is septate, conidiophores are absent. Macroconidia is sickle shaped with pointed ends and divided into 2-11 cells. Microconidia are small, round or oval shaped.

**F4** – Broad irregular hyphae that are aseptate or sparsely septate. Branching sporangiophores with columella, supporting sporangia, filled with sporangiospores. Absences of rhizoids are seen.

**IDENTIFICATION OF FUNGUS**

**F1**- *Aspergillus*

**F2**- *Pencillium*

**F3**- *Fusarium*

**F4**- *Mucor*

**TRIAL TEST**

After the trial test 2% concentration of the sample used showed high growth rate comparatively than 1% and 3%.

2% concentration was selected for the further procedures.

**PREPARATION OF CULTURE MEDIA FOR FUNGUS**

**ORANGE MEDIA**

| ORGANISM | LITTLE GROWTH | MODERATE GROWTH | HIGH GROWTH |
|---|---|---|---|
| ***Aspergillus*** | | Yes | |
| ***Pencillium*** | | Yes | |
| ***Fusarium*** | | Yes | |
| ***Mucor*** | | | Yes |

**LEMON MEDIA**

| ORGANISM | LITTLE GROWTH | MODERATE GROWTH | HIGH GROWTH |
|---|---|---|---|
| ***Aspergillus*** | | Yes | |
| ***Pencillium*** | | | Yes |
| ***Fusarium*** | Yes | | |
| ***Mucor*** | | | Yes |

**CARROT MEDIA**

| ORGANISM | LITTLE GROWTH | MODERATE GROWTH | HIGH GROWTH |
|---|---|---|---|
| ***Aspergillus*** | | Yes | |
| ***Pencillium*** | | | Yes |
| ***Fusarium*** | Yes | | |
| ***Mucor*** | | Yes | |

**PREPARATION OF CULTURE MEDIA FOR BATERIA**

**ORANGE MEDIA**

| ORGANISM | LESS GROWTH | MODERATE GROWTH | HIGH GROWTH |
|---|---|---|---|
| ***E.coli*** | | Yes | |
| ***Staphylococcus*** | | | Yes |
| ***Pseudomonas*** | | Yes | |
| ***Bacillus*** | | | Yes |
| ***Streptococcus*** | | | Yes |
| ***Klebsiella*** | | | Yes |
| ***Shigella*** | Yes | | |

**CARROT MEDIUM**

| ORGANISM | LESS GROWTH | MODERATE GROWTH | HIGH GROWTH |
|---|---|---|---|
| ***E.coli*** | Yes | | |
| ***Staphylococcus*** | | | Yes |
| ***Pseudomonas*** | | | Yes |
| ***Bacillus*** | | | Yes |
| ***Streptococcus*** | | Yes | |
| ***Klebsiella*** | | | Yes |
| ***Shigella*** | Yes | | |

**LEMON MEDIA**

| ORGANISM | LESS GROWTH | MODERATE GROWTH | HIGH GROWTH |
|---|---|---|---|
| ***E.coli*** | | Yes | |
| ***Staphylococcus*** | | Yes | |
| ***Pseudomonas*** | | | Yes |
| ***Bacillus*** | | | Yes |
| ***Streptococcus*** | Yes | | |
| ***Klebsiella*** | | | Yes |
| ***Shigella*** | Yes | | |

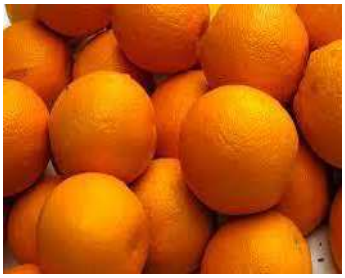

Fig: 1 - Orange

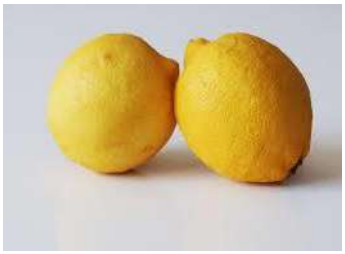

Fig: 2 - Lemon

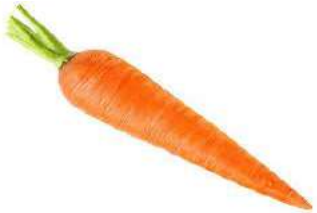

Fig: 3 – Carrot

Fig: 4 – Carrot peel powder

Fig: 5 – Lemon peel powder

Fig: 6 – Orange peel powder



Fig: 7 – Mixed fungal cultures



Fig:8- Mixed bacterial cultures

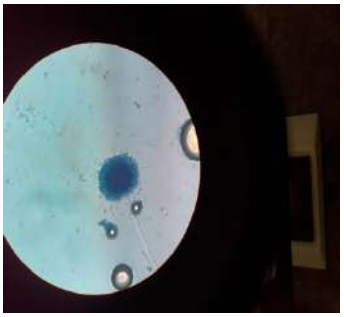
Fig: 9- LPCB *Aspergillus*

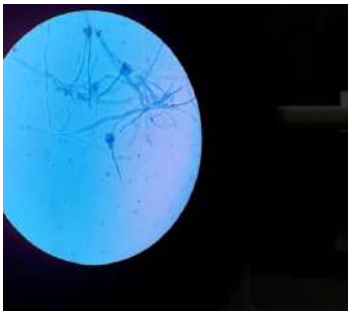
Fig: 10- LPCB *Pencillium*

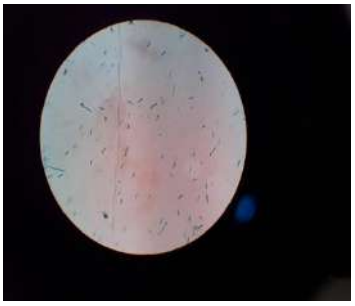
Fig: 11 – LPCB *Fusarium*

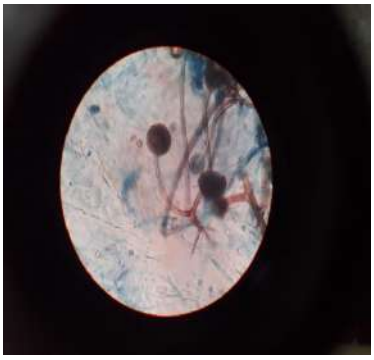

Fig: 12- LPCB *Mucor*

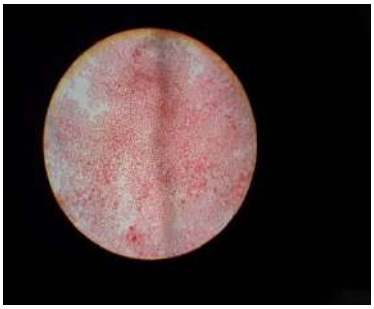

Fig: 13- Gram staining S1 (gram negative rods)

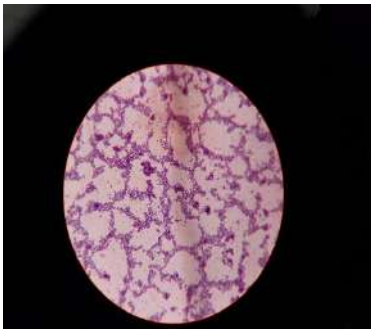

Fig: 14- Gram staining S2 (gram positive cocci)

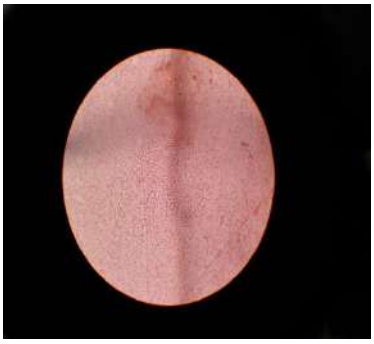

Fig: 15- Gram staining S3 (gram negative rods)

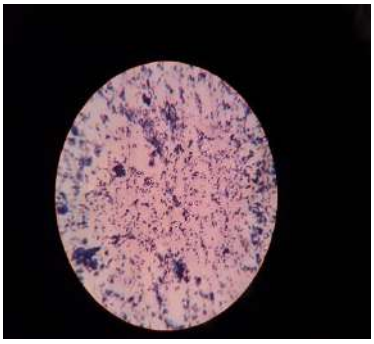

Fig: 16- Gram staining S4 (gram positive bacilli)

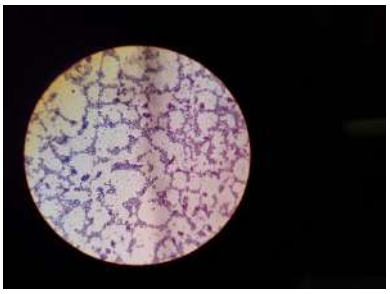

Fig: 17- Gram staining S5 (gram positive cocci)

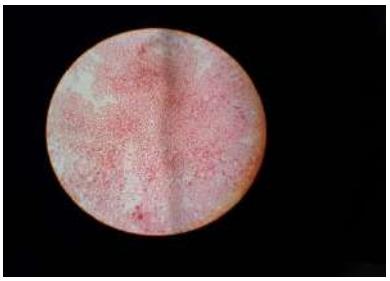

Fig: 18- Gram staining (gram negative rods)

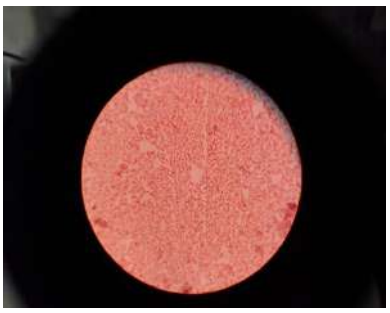

Fig: 19- Gram staining (gram negative rods)

Fig: 20- Pure culture (*Pencillium*)

Fig: 21- Pure culture (*Aspergillus*)

Fig: 22- Pure culture (*Fusarium*)

Fig: 23- Pure culture (*Mucor*)

Fig: 24- Pure culture (*Staphylococcus*)

Fig: 25- Pure culture (*E-coli, Streptococcus*)

Fig: 26- Pure culture (*Bacillus*)

Fig: 27- Pure culture (*Pseudomonas*)


Fig: 28- Pure culture (*Shigella*, *Klebsiella*)

**FUNGUS IN ORANGE MEDIA**


Fig: 29- *Pencillium*


Fig: 30- *Aspergillus*


Fig: 31- *Mucor*


Fig: 32- *Fusarium*

**FUNGUS IN LEMON MEDIA**


Fig: 33- *Pencillium*


Fig: 34- *Aspergillus*


Fig: 35- *Mucor*


Fig: 36- *Fusarium*

**FUNGUS IN CARROT MEDIUM**


Fig: 37- *Pencillium*


Fig: 38- *Aspergillus*


Fig: 39- *Mucor*


Fig: 40- *Fusarium*

**BACTERIA IN ORANGE MEDIA**


Fig: 41- *Klebsiella*


Fig: 42- *Bacillus*

Fig: 43- *E-coli*

Fig: 44- *Pseudomonas*

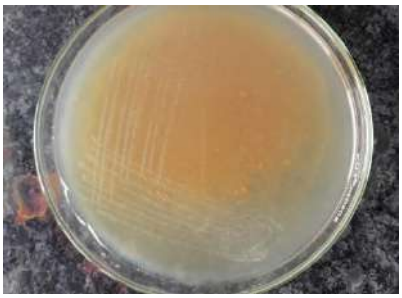

Fig: 45- *Staphylococcus*

Fig: 46- *Streptococcus*

Fig: 47- *Shigella*

**BACTERIA IN CARROT MEDIA**


Fig: 48- *Klebsiella*

Fig: 49- *Bacillus*

Fig: 50- *Pseudomonas*

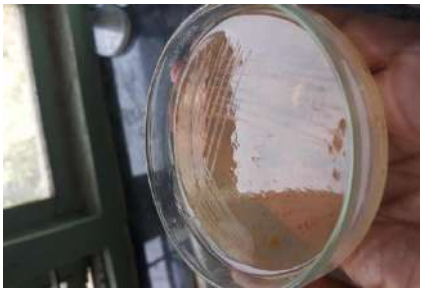

Fig: 51- *Staphylococcus*

Fig: 52- *Streptococcus*

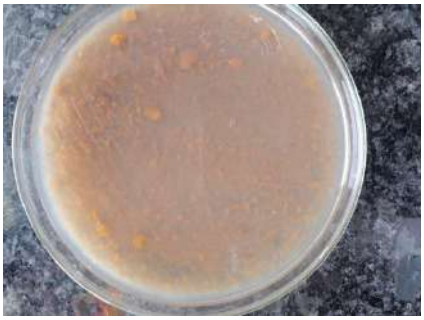

Fig: 53- *E-coli*

Fig: 54- *Shigella*

**BACTERIA IN LEMON MEDIA**

# DISCUSSION

## DISCUSSION

The new culture media can provide a cheaper and easily available media for different bacteria and fungus. These medias could be obtained in a cheaper rate compared to other culture media in the market. This project is an example of how to create wealth from waste. By this process the waste peel collected from the market can be put to use apart using them as fertilizers. Large as well as small laboratories can easily afford this media for bacterial culture and its maintenance. The media is eco-friendly and does not cause any pollution to the environment. There is no further waste generated from the production of these medias.

# CONCLUSION

## CONCLUSION

The conclusion can be made by the successful preparation of culture media for both bacteria and fungus from fruit and vegetable peel (orange, lemon and carrot respectively). After incubation in the newly made media certain bacteria and fungus showed tremendous growth were as some showed moderate and other little growth. Initially the media can provide nutrition to the organism and promote their growth. We can conclude that waste material if treated properly can be used as the resource for other living organism like bacteria and fungus to live and provide nourishment.

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# APPENDIX

# APPENDIX

## MEDIA

PEPTONE BROTH

Peptone - 20g
Sodium chloride - 5g
Starch - 1.5g
Distilled water - 1000ml
pH - 7.2

EOSIN METHYLENE BLUE

Peptone - 10g
Lactose - 5g
Sucrose - 5g
Dipotassium phosphate - 2g
Eosin - 0.4g
Methylene blue - 0.065g
Agar - 20g
Distilled water - 1000ml
pH - 7.2

MAC CONKEY AGAR

Peptone - 20g
Lactose - 10g
Bile salt - 1.5g
Sodium chloride - 5g
Neutral red - 0.03g
Crystal violet - 0.001g
Agar - 20g
Distilled water - 1000ml
pH - 7.1

NUTRIENT AGAR

Peptone - 5g

Sodium chloride - 5g

Beef extract - 3g

Yeast extract - 2g

Agar - 15g

Distilled water - 1000ml

pH - 7

SIMMON CITRATE AGAR

Ammonium hydrogen phosphate - 1g

Dipotassium phosphate - 1g

Sodium chloride - 5g

Sodium citrate - 2g

Magnesium sulphate - 0.2g

Bromothymol blue - 0.08g

Agar - 20g

pH - 6.9

MR-VP BROTH

Peptone - 7g

Dextrose - 5g

Potassium phosphate - 5g

pH - 6.9

TCBS AGAR

Peptone - 10g

Yeast extract - 5g

| | |
|---|---|
| Sodium thiosulphate | -10g |
| Sodium citrate | - 10g |
| Sodium cholate | - 3g |
| Oxgall | -5g |
| Sucrose | - 20g |
| Sodium chloride | - 10g |
| Ferric citrate | - 1g |
| Bromothymol blue | - 0.04g |
| Thymol blue | - 0.044g |
| Agar | -15g |
| pH | -8.8 |