# FRUITS PEEL WASTE AS A FUNGAL GROWTH MEDIUM

**A**

**Project work submitted to**

Department of Microbiology

Central Campus of Technology, Tribhuvan University

In Partial Fulfillment for the Award of the Degree of

Bachelor of Science in Microbiology

**By**

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# RECOMMENDATION

This is to certify that **Miss. Tara Ghimire** has completed this project work entitled **"FRUITS PEEL WASTE AS A FUNGAL GROWTH MEDIUM"** as a part of partial fulfillment of the requirements of Bachelor's degree in Microbiology under my supervision. To my knowledge, this work has not been submitted for any other degree.

……..

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**Date: 13/05/2075**

# CERTIFICATE OF APPROVAL

On the recommendation of Mr. Shiv Nandan Sah, this project of **Miss. Tara Ghimire** entitled as **"FRUITS PEEL WASTE AS A FUNGAL GROWTH MEDIUM"** has been approved for the examination and is submitted to the Tribhuvan University in Partial fulfillment of the requirements for Bachelor's degree in Microbiology.

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**……..**

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**Date: 13/05/2075**

# ABSTRACT

Most of the fruits peels are either dumped or incinerated in large extent once after the main part of fruits are used. There is an urgency to mitigate this sort of action, because at foremost, these fruits and vegetable peels are more useful for either agricultural purpose or for other different research activities in the coming future. The unused fruit peels can be simply used in different microbiological media preparing industries and in the pharmaceutical industries to some extent. The present study was aimed to formulate growth media using fruits peel waste materials such as Pineapple, Papaya, Green Banana, Yellow Banana, Orange and Pomegranate. The collected peels were air dried, grinded into fine particles using mechanical blender. Fungi isolates (*Aspergillus niger* and *Rhizopus stolonifer*) were isolated from soil and air (by serial dilution and open plate technique) using potato dextrose agar (PDA) and identified it. Similarly, *Saccharomyces cerevisiae* was isolated from *Marcha* sample. About 4.0 grams of dried fruits peel powder were added into the 100 ml of distilled water and sterilized by autoclaving. After Sterilization and cooling, one ml of fungal inoculums was added and incubated at room temperature for 5 days. The fungi growth was visually observed. *Aspergillus niger* growth was recorded in the medium containing Pineapple, Orange, Green banana, Yellow Banana and Pomegranate except Papaya. *Rhizopus stolonifer* growth was observed in the medium which contains Pineapple, Green Banana, Yellow Banana and Pomegranate except Papaya and Orange. However, the *Saccharomyces cerevisiae* growth was recorded in the medium which contains Pineapple, Green Banana, Orange and Papaya only except Yellow banana and Pomegranate.

**Keywords**: *Aspergillus niger,* Frit peel wastes, *Rhizopus stolonifer*, *Saccharomyces cerevisiae*

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| | |
|---|---|
| % | Percent |
| E.g. | Example |
| Etc. | Etcetera |
| i.e. | that is |
| Mg | milligram |
| Ml | milliliter |
| MYGP | Malt Extract, Yeast Extract, Glucose, Peptone Agar |
| PDA | Potato Dextrose Agar |
| SCP | Single Cell Protein |
| SDA | Sabouraud's Dextrose Agar |
| *Viz* | Namely |

# CHAPTER I

# INTRODUCTION

## 1.1 Background

A growth medium is a liquid or gel designed to support the growth of microorganisms. The commercially available media are very costly. Routine practical requires large amount of media on regular basis for streak plate, pour plate, and spread plate experiments. Availability of low cost media rich in nutrients, giving comparative results is the need of the day. Recent research has been focused on finding alternatives to gelling agents of media, agar in particular, and media, in general, because of its exorbitant price. (Ravimannan et al 2014)

Modern efficient agricultural practices have mobilized huge production of fruits and vegetables throughout the world. Fruits like Banana, pineapple, mango and papaya are among the most widely accepted all over the world (Jamal et al 2012). Most of the waste materials such as peels, pulp and seeds, emanating from the above-mentioned fruits cover about forty percent of the total mass. The majority of these waste materials are often randomly and improperly disposed; hence causing huge environmental disorders (Essien et al 2005; Lim et al 2014). Most of the fruit waste dumping sites provide abundant amount of necessary impetus for vectors, parasites, bacteria and fungi to thrive. A popular method to overcome or mitigate fruit waste poor handling is either landfill or incineration. However, this sort of methods may directly cause the acute air pollution and produce leachates that can easily contaminate ground water and destroy aquatic lives (Ali et al 2014)

Most of the fruits peels are either dumped or incinerated in large extent once after the main part of fruits are used. There is an urgency to mitigate this sort of action, because at foremost, these fruits and vegetable peels are more useful for either agricultural purpose or for other different research activities in the coming future. The unused fruit peels can be simply used in different microbiological media preparing labs and also in the pharmaceutical industries to some extent. Therefore, the random disposal of those fruits peels without knowing the eclectic scope of its usage should be minimized. (Gao et al 2010).

It is anticipated that the discarded fruit as well as the waste material can be utilized for further industrial processes like fermentation, bioactive component extraction, etc. There has been numerous works on the utilization of waste obtained from fruit and vegetable, dairy and meat industries. In this regard, several efforts have been made in order to utilize pineapple wastes obtained from different sources. The wastes from pineapple canneries have been used as the substrate for bromelain, organic acids, ethanol, etc. since these are potential source of sugars, vitamins and growth factors (Larrauri et al 1997; Dacera et al 2009). Several studies have been carried out since decades on trying to explore the possibility of using these wastes. In past, sugar has been obtained from pineapple effluent by ion exchange and further use it in syrup for canning pineapple slices (Beohner and Mindler, 1949). This study would try to collect and gather information regarding the utilization of pineapple wastes.

Generally, fungi are grown on Potato Dextrose Agar (PDA), Sabouraud's Dextrose Agar (SDA), Rose Bengal Agar (RBA) and Corn Meal agar (CMA) which are very expensive. Basically, every fungus requires carbon, nitrogen, and energy source to grow and survive. Utilization of agricultural waste as a substrate for fungal cultures for the production value added products has been reported by observing some works done on it. Therefore, fruits peel wastes may meet requirements and work as fungal growth medium and can convincingly replace expensive media in the market. This will add a benefit of minimal contamination in the cultures as it does not meet the needs of every microbe. Fruits peel wastes has been exploited for the production of many high value products but its potential as fungal growth medium has never been reported. Therefore, the main aim of this study will be designing a cost effective medium for some of the fungal cultures, that is, *Aspergillus niger, Rhizopus stolonifer* and *Saccharomyces cerevisiae* using fruit peels wastes as raw material. (Saranraj and Anbu, 2017)

## 1.2 Rationale of the study

People have been using fruits and its products for since a very long time and the knowledge of fruits as a source of energy and as medicine has been transferred from generation to generation. However, in past and early present century the use of fruits has been common for any sorts of occasion. Beside mere purposes, the use of peels and seeds of most of the fruits has not been in practice. Now, approach that is much more scientific is to be made to determine the use of peels and seeds of fruits which are going useless. Similarly, reasoning is to be done and applications are to be determined for using those peels in the field of microbiology. Nowadays, due to the shortage and price hike of the commonly used microbial media, there is a necessity for the discovery of new source of nutrient media in scientific way by using the available raw materials that is used in our daily life. As fruit peels are not mostly used, this finding will support for scientific validation for the advance use of those peels for the productions of fungal media. It can also be used as pharmaceuticals and microbial media producing industries.

## 1.3 Objectives

### 1.3.1 General objective

- To study the use of fruits peel waste as a novel media for the growth of economically important fungi.

### 1.3.2 Specific objectives

- To prepare fine powder of the peels of Pineapple, Papaya, Yellow banana, Green banana, Orange and Pomegranate.
- To isolate and identify the fungal isolates by soil dilution, open plate technique and *Marcha* sample using Potato Dex-trose Agar.
- To prepare fungal growth media using fine powder of fruits peels.
- To culture fungal isolates on media prepared from fruits peels.

## 1.4 Limitations of the study

In this study, the scope of usage of fruits peels as the novel media for the growh of some fungi was tested on laboratory scale around the short time period. Thus, this study does not guarantee for its direct usage in any microbiology, pharmaceutical industries. The main hindrance behind the direct use of fruit peels as the media for the growth of fungi in any microbiology or pharmaceutical laboratory is the presence of phytochemicals such as tannins, alkaloids, flavonoids, steroids, essential oils and minerals present in fruits peels. These oils and phytochemicals may act as an inhibiting agent and may cause the hindrance in the metabolism and growth process. Thus, scientific and systematic synthesis of fungal growth media on large scale by the usage of fruits peels by maintaining all necessary pre-requisite is somewhat a matter of urgency in the present context.

# CHAPTER II
# LITERATURE REVIEW

## 2.1 Fruit Peels

Tropical and subtropical fruits processing have considerably higher ratios of by-products than the temperate fruits. Fruit by-products are not exceptions and they consist of the residual pulp, peels, stem and leaves. The increasing production of fruits processed items, results in massive waste generations. This is mainly due to selection and elimination of components unsuitable for human consumption (Nunes et al 2009).

Besides, rough handling of fruits and exposure to adverse environmental conditions during transportation and storage can cause up to 55 percentages of product waste. These wastes are usually prone to microbial spoilage thus limiting further exploitation. Further, the drying, storage and shipment of these wastes is cost effective and hence efficient, inexpensive and eco-friendly utilization is becoming more and more necessary (Nunes et al 2009).

Peel, also known as rind or skin, is the outer protective layer of a fruit or vegetable. Botanically, the rind is usually the exocarp, which includes the hard shell in fruits such as nuts. Depending on the thickness and taste, peel is sometimes eaten as part of the fruit, as seen with apples. In some fruits such as banana, the peel is unpleasant or inedible; thus, it is removed and discarded (Schieber et al 2001).

Most fruit peels are discarded as waste after the inner fleshy portions have been eaten. It is vital that peels be removed from most fruits before eating; and more importantly before using them in fruit juice industries to prevent contamination. Processing of fruits into juices reduces and prevents wastage when fruits are in season (Oladiji et al 2010).

The fruit juice is the next best thing to fresh fruit, and can be packaged in aseptic, easily transportable containers that are less susceptible to damage and have a relatively long storage life. Juice extraction and separation therefore open up

new market opportunities for tailoring fruit products to modern consumer demands (Olukunle et al 2007).

At the time of producing fruit juice, a lot of peels are produced. This could cause environmental pollution and health problems if left untreated. Peels can be removed manually, mechanically and by the use of enzymes. A lot of money, time, equipment and other resources are used to remove the peels in the industry. Enzymatic removal of peels could be cheaper and more effective than manual and mechanical methods. The use of enzyme in the manufacture of various industrial products is wide spread (Forgatty and Kelly 2013). Development of microbes that will synthesize these enzymes will then be useful to man.

## 2.2 Fungi

A fungus (plural: fungi or funguses) is any member of the group of eukaryotic organisms that includes microorganisms such as yeasts and molds, as well as the more familiar mushrooms. These organisms are classified as a kingdom, Fungi, which is separate from the other eukaryotic life kingdoms of plants and animals. A characteristic that places fungi in a different kingdom from plants, bacteria, and some protists is chitin in their cell walls. The carbohydrates stored in fungi is in the form of glycogen. The 'fruit' body of fungus is only seen, while the living body of the fungus is a mycelium, it is made of tiny filaments called hyphae. The mycelium is hidden. Nutrition in fungi is by absorbing nutrients from the organic material in which they live. Fungi do not have stomachs; they digest their food before it passes through the cell wall into the hyphae. The hyphae secrets enzymes and acids that break down the organic material into simple compounds.

Similar to animals, fungi are heterotrophs; they acquire their food by absorbing dissolved molecules, typically by secreting digestive enzymes into their environment. Fungi do not photosynthesize. Growth is their means of mobility, except for spores (a few of which are flagellated), which may travel through the air or water. Fungi are the principal decomposers in ecological systems.

## 2.3 Classification of fungi

The kingdom Fungi contains five major phyla that were established according to their mode of sexual reproduction or using molecular data. Fungi are mainly classified as Chytridiomycota (Chytrids), the Zygomycota (conjugated fungi), the Ascomycota (sac fungi), the Basidiomycota (club fungi) and the recently described Phylum Glomeromycota. The Deuteromycota is an informal group of unrelated fungi that all share a common character – they use strictly asexual reproduction.The fungi I have used for my study are describe shortly below:

### 2.3.1 *Rhizopus stolonifer*

*Rhizopus stolonifer* is commonly known as black bread mold. It is a member of Zygomycota and considered the most important species in the genus *Rhizopus*. It is one of the most common fungi in the world and has a global distribution although it is most commonly found in tropical and subtropical regions. It is a common agent of decomposition of stored foods. Like other members of the genus *Rhizopus*, *R. stolonifer* grows rapidly, mostly in indoor environments.

It is found on all types of mouldy materials. It is often one of the first molds to appear on stale bread. It can exist in the soil as well as in the air. Varieties of natural substrata are colonized by this species because *R. stolonifer* can tolerate broad variations in the concentration of essential nutrients and can use carbon and nitrogen combined in diverse forms. In the laboratory, this fungus grows well on different media, including those that contain ammonium salts or amino compounds. Colonies are fast growing and cover an agar surface with a dense cottony growth that is at first white becoming grey or yellowish brown with sporulation.

### 2.3.2 *Aspergillus niger*

*Aspergillus niger* is a member of the genus *Aspergillus* which includes a set of fungi that are generally considered asexual, although perfect forms (forms that reproduce sexually) have been found. *Aspergillius* are ubiquitous in nature. They are geographically widely distributed, and have been observed in a broad range of habitats because they can colonize a wide variety of substrates.

*Aspergillus niger* is commonly found as a saprophyte growing on dead leaves, stored grain, compost piles, and other decaying vegetation. The spores are widespread, and are often associated with organic materials and soil. The primary uses of *Aspergillus niger* are for the production of enzymes and organic acids by fermentation. *Aspergillus niger* is also used to produce organic acids such as citric acid and gluconic acid (Ravimannan et al 2014).

*Aspergillus niger* is a ubiquitous fungus that grows very quickly. Strains can be isolated from many different ecological habitats such as soil, plant debris, rotting fruit, and even indoor air environments. Macroscopically, this fungus can be identified growing on substrates producing colonies of felt like yellow to white hyphae, turning black with the formation of conidia. Microscopically, *Aspergillus niger* can be identified by its hyaline, septate hyphae. Asexual conidiophores can be identified by being long and globose at the tip, with what appears to be a hymenial layer of structures, each "ejecting" its own spore (Ravimannan et al 2014).

### 2.3.3 *Saccharomyces cerevisiae*

*Saccharomyces cerevisiae* is a eukaryotic microbe. More specifically, it is a globular-shaped, yellow-green yeast belonging to the Fungi kingdom, which includes multicellular organisms such as mushrooms and molds. Most often it is found in areas where fermentation can occur, such as the on the surface of fruit, storage cellars and on the equipment used during the fermentation process. *Saccharomyces cerevisiae* is famously known for its role in food production. It is the critical component in the fermentation process that converts sugar into alcohol; an ingredient shared in beer, wine and distilled beverages. It is also used in the baking process as a leavening agent; yeast-releasing gas into their environment results in the spongy-like texture of breads and cakes. Because of its role in fermentation, humans have known about and used *Saccharomyces cerevisiae* for a long time (Mondal et al 2012).

## 2.4 Fruit peel wastes and fungal growth

fruit production of world is around 256 million metric tons (MMT). China and India are the top nations producing wide ranges of fruits and exporting to all over the world. Large varieties of fruits are grown in Nepal also, of which mango, banana, orange, guava, grape, pineapple and apple are the major ones. Apart from these, fruits like papaya, banana, jackfruit, pomegranate grown in tropical and sub-tropical areas and peach, pear, almond, walnut, apricot and strawberry in the temperate areas. Different fruits are grown throughout the country based on the different seasons.

Improved fruit and vegetable production through efficient agricultural practices mobilize huge investments in fruit and vegetable processing across the world. Banana, pineapple, pomegranate and papaya are among the most widely acceptable fruits planted on commercial level worldwide (Jamal et al 2012). Waste generation through these fruits is on the increase due to sustained surge in world population, improved economic growth in developing nations and improved access to nutrition education in high fruit producing countries.

Wastes emanating from aforementioned fruits include peels, pulp and seeds that constitute about 40 % of the total mass of each fruit. The majority of these waste materials were often improperly disposed; hence constitute huge environmental disorders (Essien et al 2005; Lim et al 2010). Fruit waste dumping sites provide necessary impetus for vectors, pathogenic bacteria and yeast to thrive. A popular approach to mitigating fruit waste poor handling is landfill and incineration. These methods orchestrate an acute air pollution problem by generating massive leachates that contaminate ground water and destroy aquatic lives (Taskin et al 2010; Ali et al 2014).

The cost of all the microbiological media is rising at a fast pace. To tackle this problem some new microbiological media should be designed which are efficient as well as cost effective. This may be achieved by using agricultural wastes as raw materials for microbial media. Utilization of agricultural waste as a substrate for fungal cultures for the production of value added products has been reported which includes cellulase production by some fungi cultured on pineapple waste (Omojasola et al 2008). Carotenoids production is carried out

on agricultural waste using *Blakeslea trispora* and cellulase enzyme production on agricultural waste by *Aspergillus niger* (Milala et al 2005). Sugarcane bagasse has been also reported as an energy source for the production of lipase by *Aspergillus fumigatus* (Naqvi et al 2013).

Generally, fungi are grown on Potato dextrose agar (PDA), Sabouraud's dextrose agar (SDA), Rose Bengal Agar (RBA) or Corn Meal agar (CMA) which are very expensive. Basically, every fungus requires carbon, nitrogen and energy source to grow and survive. Fruit peel wastes may meet these requirements and work as a fungal growth medium and can replace expensive media in the market. This will add a benefit of minimal contamination in the cultures as it does not meet the needs of every microbe. Fruit peel wastes has been exploited for the production of many high value products but its potential as fungal growth medium has never been reported. The aim of the current study was to design a cost effective and efficient medium for fungal cultures, that is, *Aspergillus niger*, *Rhizopus stolonifer* and *Saccharomyces cerevisiae* using fruit peel wastes as raw material.

## 2.5 Utilization of fruit peel wastes for the cultivation of fungi

According to India Agricultural Research Data Book 2016, the total waste generated from fruits and vegetables comes to 50 million tons per annum. Fruit wastes rich in carbohydrate content and other basic nutrients could support microbial growth. Thus, fruit processing wastes are useful substrates for production of microbial proteins. The utilization of fruit wastes in the production of SCP (Single Cell Protein) will help in controlling pollution and also in solving waste disposable problem to some extent in addition to satisfy the world shortage of protein rich food.

It is anticipated that the discarded fruit as well as the waste material can be utilized for further industrial processes like fermentation, bioactive component extraction, etc.It is also done for waste recycling of fruits peels. There has been numerous works on the utilization of waste obtained from fruit and vegetable, dairy and meat industries. In this regard, several efforts have been made in order to utilize pineapple wastes obtained from different sources. The wastes from pineapple canneries have been used as the substrate for bromelain, organic

acids, ethanol, etc. since these are potential source of sugars, vitamins and growth factors (Larrauri et al 1997, Nigam 199, Dacera 2009). Several studies have been carried out since decades on trying to explore the possibility of using these wastes. In past, sugar has been obtained from pineapple effluent by ion exchange and further use it in syrup for canning pineapple slices (Beohner and Mindler 1949). This paper would try to collect and gather information regarding the utilization of pineapple wastes

Mondal et al (2012) used cucumber and orange peels to evaluate the production of single cell protein using *Saccharomyces cerevisiae* by submerged fermentation. The authors state that the bioconversion of fruit wastes into single cell protein production has the potential to solve the worldwide food protein deficiency by obtaining an economical product for food and feed. Fruit wastes rich in carbohydrate content and other basic nutrients could support microbial growth. Kondari and Gupta (2012) used Apple, turnip, papaya and banana peels for alcohol fermentation and biomass production. The use of legume seeds as alternative nutrient media for bacteria and fungi has been reported (Tharmila et al 2011; Arulanantham et al 2012; Ravimannan et al *2014*).

Generally, growth media for fungi contain carbon and nitrogen sources, and most fungi require several specific elements for growth and reproduction (Walker and White 2005; Gao et al 2007). Cultural medium is defined as any material in which microorganism find nourishment for growth and development. Fungi, like any other living organism, require nutrients for their life processes. This is obvious from the fact that they feed on varieties of food substances (Hawker and Alan 1979). Investigation into the composition of culture media has established that the important ingredients such as nitrogen, carbon (a source of energy) vitamins and growth factors, mainly essential mineral salts are required for fungal growth (Ruth et al 2012). Different researchers (Weststeijn and Okafor 1971; Adesemoye and Adedire, 2005; Tharmilla and Thavaranjit 2011) have studied the feasibility of developing alternative media for cultivation of fungi apart from the conventional ones like Sabouraud's Dextrose Agar (SDA) and Potato Dextrose Agar (PDA).

Apple, orange, banana and other fruits locally available and thus serve as readily available raw materials for the separation of ethanol yeasts. Bansal and Singh (2003) isolated various strains of indigenous yeasts capable of producing ethanol from local fermented pineapple juiced. Hossain et al (2014) did comparative study on ethanol production from molasses using *Saccharomyces cerevisiae* and *Zymomonas mobilis*.

Fruit waste contains many reusable substances of high value. The wastes from canneries have high exploitation potential with encouraging future. Furthermore, dietary fibers and phenolic antioxidants could be used as impending nutraceutical resource, capable of offering significant low-cost nutritional dietary supplement for low-income communities. The booming market of functional food has created a mammoth vista for utilization of natural resources. (Ruth et al 2012).

If novel scientific and technological methods are applied, valuable products from fruit wastes could be obtained. In this regard, cheap substrates, such as pineapple wastes have promising prospect. Thus, environmentally polluting by-products could be converted into products with a higher economic value than the main product. However, verification of this hypothesis is indispensable in order to apply fruit cannery waste as fungal growth medium (Ruth et al 2012).

# CHAPTER III
# MATERIALS AND METHODS

## 3.1 Materials Used

The materials used in this research is mentioned in Appendix-A

## 3.2 Population and Sample

**I) Fungal sample***: Asergillus niger* and *Rhizopus stolonifer* were isolated from soil and air by serial dilution and open plate technique and identified by Lactophenol Cotton Blue staining. Meanwhile, *Saccharomyces cerevisiae* was isolated from *Marcha* sample and identified by wet mount microscopy.

**II) Raw materials:** Common fruits like green banana, yellow banana and Papaya were collected from home garden, while other fruits i.e. Orange, Pineapple and Pomegranate were bought from local market of Tarahara.

## 3.3 Site of the study

The intended work on the usage of its peels as the novel media for fungal growth was carried out in the microbiology lab of Central Campus of Technology, Hattisar, Dharan. The laboratory was provided with all the necessary materials and equipment that were required for this study.

## 3.4 Research method

The method for this study was qualitative analysis of growth of fungi in fruit peel media. This study was based on the culture method.

## 3.5 Type of study

The study was of descriptive type

## 3.6 Work flow-chart

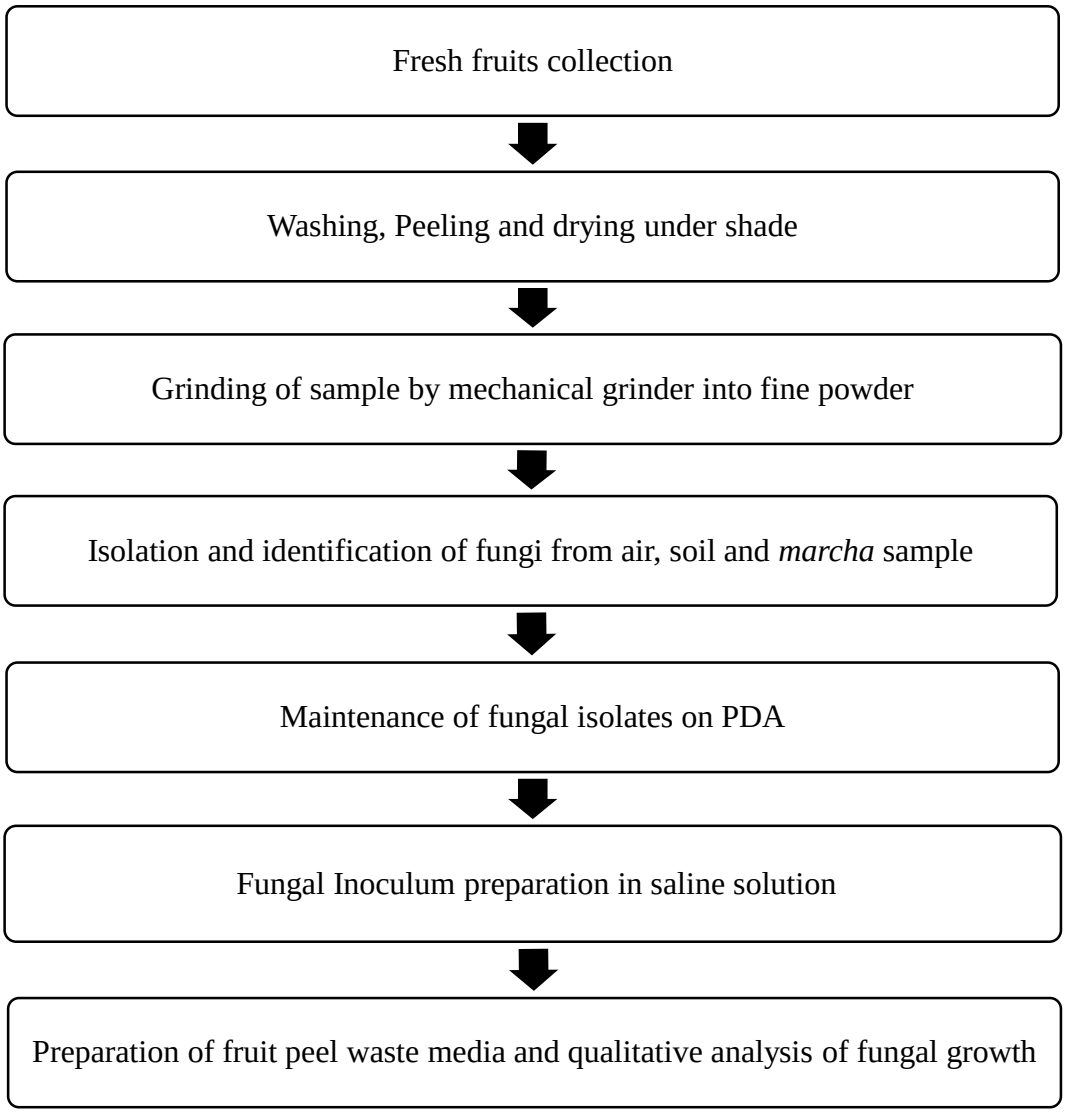


**Fig: Work flow-chart of the study**

## 3.7 Fruit collection and peel waste preparation

The first step of the experiment involved the preparation of dry powder from fruit peels. For this, fresh fruits were collected from house garden and some of the fruits were brought from the local market of Tarahara. The fruits were then thoroughly washed. After washing, they were peeled and then the peeled part of the fruit i.e. outermost layer of fruit, were air dried at room temperature for 10 days to remove the moisture. Fruit peels might get discolored and even lose its important minerals and vitamins during direct sun drying, so best dried under shade. The dried peels were then powered by mechanical grinder and sieved to give powdery form. The powder was stored in polythene bag at room temperature.

## 3.8 Isolation and identification of fungi

One of the most important part of this experiment is to isolate and identify the fungi required for the further experimentation on fruit peel media. Firstly, fungi (*Aspergillus niger and Rhizopus stolonifer)* were isolated from air and soil by open plate and serial dilution technique. On the other hand, *Saccharomyces cerevisiae* was isolated from *marcha* sample.

The potato dextrose agar (PDA) plates were prepared in lab and were plated aseptically. Some plates were then opened in lab and other were opened near the toilet site in college premises. All of the plates were left opened for around 15 minutes. Lastly, the plates were incubated at room temperature for 7-10 days. To acquire the proper and well grown colony, fungi were also isolated from soil sample by serial dilution. For this, different soil samples from different places of college premises were collected. One grams of soil sample was then serially diluted upto $10^{-4.}$ 0.1ml of sample was pipetted and spread plated onto PDA media plate. The plates were incubated for 5-7 days at 28$^{o}$C.

On the other hand, *Saccharomyces cerevisiae* was directly isolated from *marcha* sample. *Marcha* was bought from the market and was used as the substrate for isolating yeast through it. *Marcha* sample was crushed into fine powder and 1 gram of *marcha* sample was then serially diluted upto $10^{-2}$. Successive dilutions were made and lastly 0.1ml of sample were pipetted from

different diluted tubes and were spread plated in MYGP agar plates. Lastly, the plates were incubated at $28^0$C for 7-10 days.

Another pivotal and most crucial stage of this experiment is the identification of desired fungi. Fungi *viz. Aspergillus niger* and *Rhizopus stolonifer* were identified by Lactophenol Cotton Blue (LPCB) staining technique. In this technique, few drops of lacto-phenol cotton blue stain were dropped on a clean and dry slide. After that, the selected fungi was placed onto the stain with the help of sterile forcep, needles and teased properly.The fungal isolates were covered by cover-slip and observed under 40x objective lens. Meanwhile, *Saccharomyces cerevisiae* was observed under microscope without following any staining technique. Wet mount of fungal sample was made and observed directly under microscope. At the end, the test sample was covered by cover-slip and was observed under the microscope. Based on their mycelial feature, fruiting body and structure the selected fungal colonies were identified.

## 3.9 Maintenance of fungal isolates

After identifying the fungi, the fungal colonies were sub-cultured in freshly prepared PDA and MYGP agar plates. For this, fungal isolates which were selected to observe under microscope were selected as the base culture for sub culturing it as a new colony. Fungal isolates were pricked with the help of inoculating loop and the loop was streaked gently over the fresh media plates aseptically. Lastly, the media plates with the isolates were incubated at room temperature for 5 days. The isolated culture was maintaining at sand-soil-calcium carbonate (78:20:2) for preserving culture for a long time.

## 3.10 Inoculum preparation

The suspension of 4 days old cultures of fungi (*Aspergillus niger*, *Rhizopus stolonifer* and *Saccharomyces cerevisisae*) were used to study the qualitative and quantitative growth analysis. They were prepared in saline solution (0.85 % sodium chloride). The fungal cultures were inoculated into 50 ml of saline and incubated at room temperature for 5 hours. Saline solution with lower concentration helps fungi in their metabolism and growth process. Similarly, saline solution helps to maintain and balance the osmotic pressure of growth media too.

## 3.11 Preparation of fruit peel waste media

About 4.0 grams of dried fruit peel powder were added into the 100 ml of distilled water and sterilized by autoclaving at 121°lbs/pressure for 15 minutes. After sterilization, the fruit peel waste broths were cooled and then 1ml of fungal inoculums were transfer to it.

## 3.12 Qualitative analysis of fungal growth

The inoculum added broths were incubated at room temperature for 5-7 days. The presence/absence of fungal growth in fruit peel media was visually observed.

# CHAPTER IV

# RESULTS

The fruit samples were collected from local market of Tarahara and some fruits were also collected from house garden. Fruits were thoroughly washed and peeled. The peels were air dried under shade and blended to fine powder.

Fungal samples were isolated from air, soil and *marcha* sample. Since the fungal grew in cluster, it was bit difficult to identify them. The fungal colonies were identified by Lacto Phenol Cotton Blue staining technique and were sub cultured in entirely fresh media plates. Those sub-cultured fungi were taken as the test organism to carry out the further experimentation.

## 4.1 Colonial characteristics of fungal isolates

Different types of fungal colonies were isolated in Potato Dextrose Agar and MYGP agar plates initially. In order to get proper information on fungal colonies and to isolate the better fungal colony, the well- grown fungal colonies were identified and subcultured on respective agar plates i.e. *Aspergillus niger* and *Rhizopus stolonifer* in PDA and *Saccharomyces cerevisiae* in MYGP agar plates. The three fungi sub-cultured on agar media have significant colonial characteristics which are mentioned in Table 1.

**Table 1:** Colonial characteristics of fungal isolates on PDA and MYGP agar plates

| S.N | Media used | Colony morphology | Probable identity |
|---|---|---|---|
| 1 | PDA | Velvety, black, creamy | *Aspergillus niger* |
| 2 | PDA | Colonies grow rapidly, resemble cotton candy. Turned into blackish colony due to ageing. | *Rhizopus Stolonifer* |
| 3 | MYGP | Flat, smooth, moist, glistening and cream in color. | *Saccharomyces cerevisiae* |

## 4.2 Qualitative growth analysis of fungi in fruit peel waste media

The effect of six different fruit peel media *viz*., pineapple, papaya, orange, yellow banana, green banana and pomegranate on the qualitative growth of *Aspergillus niger*, *Rhizopus stolonifer* and *Saccharomyces cerevisiae* were studied and the results were given in Table 2. It was observed that the *Aspergillus niger* growth was recorded in the medium which contains pine apple, pomegranate, orange, yellow banana and green banana. The *Aspergillus niger* growth was not recorded in the medium containing papaya. *Rhizopus stolonifer* growth was noticed in the medium that contains pine apple, green banana, yellow banana and pomegranate. The *Rhizopus stolonifer* growth was not recorded in the medium containing papaya and orange. However, the *Saccharomyces cerevisiae* growth was recorded in the medium which contains papaya, pineapple, orange and green banana. The *Saccharomyces cerevisiae* growth was not recorded in the medium containing yellow banana and pomegranate.

**Table 2:** Qualitative growth analysis of fungal in fruit peel wastes

| S.N | Organism | Papaya | Pine apple | Pome-granate | Yellow banana | Green banana | Orange |
|---|---|---|---|---|---|---|---|
| 1 | *Aspergillus niger* | _ | + | + | + | + | + |
| 2 | *Rhizopus stolonifer* | _ | + | + | + | + | _ |
| 3 | *Saccharomy-ces cerevisiae* | + | + | _ | _ | + | + |

**[Note: (+) = Positive growth; Nil Growth = (-)]**

# CHAPTER V

# DISCUSSION

Tropical and subtropical fruits processing have considerably higher ratios of by-products than the temperate fruits. Fruit by-products are not exceptions and they consist of the residual pulp, peels, stem and leaves. The increasing production of pineapple processed items, results in massive waste generations. This is mainly due to selection and elimination of components unsuitable for human consumption. Besides, rough handling of fruits and exposure to adverse environmental conditions during transportation and storage can cause up to 55 % of product waste. These wastes are usually prone to microbial spoilage thus limiting further exploitation. Further, drying, storage and shipment of these wastes is cost effective and hence efficient, inexpensive and eco-friendly utilization is becoming more and more necessary. (Nunes et al 2009)

The commercially available media are very costly. Routine practical requires large amount of media on regular basis for streak plate, pour plate and spread plate experiments. Availability of low cost media providing rich in nutrients is much warranted. The search for alternative, cheap media for use in laboratory agents for routine microbiological experiments is going on. Recent research has been focused on finding alternatives to gelling agents of media, agar in particular, and media, in general, because of its exorbitant price (Ravimannan et al 2014)

Waste utilization in fruits and vegetable processing industries is one of the important and challengeable jobs around the world. It is anticipated that the discarded fruits as well as its waste materials could be utilized for further industrial purposes viz. fermentation, extraction of bioactive components, extraction of functional ingredients etc. Fruit waste contains many reusable substances of high value and novel scientific technological methods are applied, valuable products from fruit wastes could be obtained. In this regard, cheap substrates, such as fruit peel wastes have promising prospect. Thus, environmentally polluting by-products could be converted into products with a higher economic value than the main product.

Fruit peel wastes may meet these requirements and work as a fungal growth medium and can replace expensive media in the market. This will add a benefit of minimal contamination in the cultures as it does not meet the needs of every microbe. Fruit peel wastes has been exploited for the production of many high value products but its potential as fungal growth medium has never been reported. The aim of the current study was to design a cost effective and efficient medium for fungal cultures, that is, *Aspergillus niger, Rhizopus stolonifer* and *Saccharomyces cerevisiae* using fruit peel wastes as raw material.

Fresh fruits were brought from local market of Tarahara. Then, they were subsequently washed peeled, and the peels were dried under shade. According to (Gyawali, 2013) immediate drying prevents microbial fermentation and degradation of metabolites. In addition, protection from direct sunlight is essential to minimize chemical reactions induced by ultra violet rays. The sample was grinded to powdery form using mechanical blender. Fungal samples were isolated from air, soil and *marcha* sample. *Aspergillus niger* and *Rhizopus stolonifer* were isolated from air by open plate technique and from soil by serial dilution technique in PDA media plates. Similarly, *Saccharomyces cerevisiae* was isolated from *marcha* sample using MYGP media. Subculture of fungal colony was done after identifying the suspected fungal colony by lacto phenol cotton blue staining. A loopful of sub cultured fungal colony was incubated in 0.85% of saline solution at room temperature. Lastly, the saline solution was poured into the flask containing autoclaved and sterile fruit peel solution (peel powder in distilled water). Qualitative growth analysis of respective fungi in different fruit peel media were made. (P Saranraj and S Anbu. 2017).

Agricultural waste materials support the good growth of fungi. Microbiological studies depend on the ability to growth and maintain microorganisms under laboratory conditions by providing suitable culture media that offer favourable conditions (Domsch and Anderson 1980). The nutrients in the wastes included protein, carbohydrate and minerals. Protein constitutes a significant portion of microbial cells and thus is necessary for the growth of microorganisms (Prescott and Harley 2002). The protein content of the formulated media must have ensured a good supply of nitrogen while the

carbohydrate content served as additional carbon source both of which are essential for good fungal growth. The mineral content of the wastes in the formulated media was probably useful for some aspects of the fungi's metabolism. Although, all organisms for their life processes require moisture (water) and fungi in particular require water for extracellular digestion of nutrients (Pelczar et al 1993), the moisture content of each of the samples has negligible or no effect on the growth of fungi tested because they were grown in the media containing water. In terms of mean radial growth, Sweet Potato Peel Agar was found to be the best media for growing three (*Aspergillus niger, Geotrichum candidum* and *Saccharomyces cerevisiae*) out of the four fungi. It thus produced the highest growth rates in these three fungi. Most fruit peels are discarded as waste after the inner fleshy portions have been eaten. It is vital that peels be removed from most fruits before eating; and more importantly before using them in fruit juice industries to prevent contamination. Processing of fruits into juices reduces and prevents wastage when fruits are in season. (Oladiji et al. 2010).

Fruit waste contains many reusable substances of high value. The wastes from canneries have high exploitation potential with encouraging future. Furthermore, dietary fibers and phenolic antioxidants could be used as impending nutraceutic resource, capable of offering significant low-cost nutritional dietary supplement for low-income communities. The booming market of functional food has created a mammoth vista for utilization of natural resources. If novel scientific and technological methods are applied, valuable products from fruit wastes could be obtained. In this regard, cheap substrates, such as pineapple wastes have promising prospect. Thus, environmentally polluting by-products could be converted into products with a higher economic value than the main product. However, verification of this hypothesis is indispensable inorder to apply fruit cannery waste as industrial raw materials (P Saranraj and S Anbu. 2017).

# CHAPTER VI

# CONCLUSION AND RECOMMENDATIONS

## 6.1 Conclusion

This present study has revealed that the fruit peel waste materials contain minerals and nutrients that can meet the nutritional requirements of industrially important fungi. Thus, they can be utilized as alternative materials in the formulation of culture media for the in vitro cultivation of fungi for industrial and research purposes. An important advantage of the fruit peels used in formulating the various media is that it is readily available in Nepal. Readily available fruits like pineapple, pomegranate, orange, green banana, yellow banana and papaya can be taken as the base for formulating cost effective and useful fungal media. This study has shown that fungi such as *Aspergillus niger, Rhizopus stolonifer* and *Saccharomyces cerevisiae* can be grown in fruit peels also. Thus, this study helps to mitigate the cost burden for producing fungal growth media. In solving the problem of the shortage of culture media for laboratory practical, the result of this research will go a long way in ameliorating this problem. Further research is still needed in the application of modern tools and methods in the study of fungal physiology as this will assist in manipulation of waste materials into useable forms.

## 6.2 Recommendations

- Research can be performed to determine its constituents, their uses in growing different microorganisms such as bacteria and other fungi.
- Due to time limitation, only few samples were mobilized in this study. For more conclusive result, the number of samples can be increased.
- Standard and well maintained test and lab works need to be done to ensure the use of fruit peels in formulating qualitative microbial growth media.
- Microbial growth media producing companies like Himedia Pvt Ltd and others should give more emphasis on using agro waste, fruit and vegetable peels in formulating cost effective growth media.

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# APPENDICES

## APPENDIX A: MATERIALS USED

**Glasswares**

Pipettes

Test tubes

Petri plates

Conical flask

Beaker

Glass rod

**Equipments:**

Autoclave

Hot air oven

Microscope

Incubator

Refrigerator

**Chemicals:**

Ethanol

Lysol

Sodium chloride (Nacl)

**Materials:**

Wash bottle

Burner

Markers

Price tag

**Sample:**

*Marcha*

Soil sample

Fruits (Pineapple, Pomegranate, Papaya, Orange, Green banana, Yellow banana)

**Others:**

Distilled water

Spatula

Digital balance

# APPENDIX B: COMPOSITION OF MEDIA USED

## I) Potato Dextrose Agar

| Ingredients | Grams/Liter |
|---|---|
| Potatoes infusion | 200 g |
| Dextrose | 20 g |
| Agar | 15 g |
| Final pH **(**at 25$^{o}$C**)** | 5.6±0.2 |

## II) Malt, Yeast Extract, Glucose, Peptone Agar (MYGP Agar)

| Ingredients | Grams/Liter |
|---|---|
| Yeast extract | 3.0 g |
| Malt extract | 3.0 g |
| Gelatine Peptone | 5.0 g |
| Dextrose | 10 g |
| Cupric Sulphate | 0.4 g |
| Agar | 20 g |
| Final pH | 6.2±0.2 |