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Fruits peel waste as a novel media for the growth of economically important Fungi

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Abstract
The present study was aimed to formulate growth media using fruits peel waste materials such as Pine apple, Mango, Jack fruit, Green Banana, Yellow Banana, Sweet Lime and Pomegranate. The fresh fruits were collected from local market of Tirupattur, Tamil Nadu and the peels were used for the study. The peels were air dried, grinded into fine particles using mortar and pestle and then sieved with 1mm sieve size. Fungi isolates (*Aspergillus niger, Rhizopus stolonifer* and *Penicillium chrysogenum*) were isolated from spoilt bread and orange using potato dextrose agar (PDA) and identified. About 4.0 grams of fruit peel wastes were added into the 100 ml of distilled water and sterilized by autoclaving at 121°C for 15 minutes. After Serilization, the fruit peel waste broths were cooled and then one ml of industrially important fungal inoculums was added. The inoculums added broths were incubated at room temperature for 3 days. The presence/absence of the fungi growth was visually observed.

A. niger growth was recorded in the medium containing Pine apple, Mango, Jack fruit and Green banana. The *A. niger* growth was not recorded in the medium containing Yellow banana, Sweet lime and Pomegranate. *R. stolonifer* growth was observed in the medium which contains Pine apple, Mango, Sweet lime and Pomegranate. The *R. stolonifer* growth was not recorded in the medium containing Yellow banana, Jack fruit and Mango. However, the *P. chrysogenum* growth was recorded in the medium which contains Pine apple, Mango, Sweet lime and Pomegranate. The *P. chrysogenum* growth was not recorded in the medium containing Yellow banana, Jack fruit and Mango.

Keywords: Fruit peel wastes, Fungi, *Aspergillus niger, Rhizopus stolonifer* and *Penicillium chrysogenum*

Introduction
Modern efficient agricultural practices mobilize huge productions of fruit and vegetables throughout the world. Banana, pineapple, mango and papaya are among the most widely acceptable fruits (Jamal *et al*., 2012) [6]. Wastes emanating from aforementioned fruits include peels, pulp and seeds that constitute about 40 % of the total mass. The majority of these waste materials was often improperly disposed; hence constitute huge environmental disorders (Essien *et al*., 2005; Lim *et al*., 2010) [2]. 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 (Ali *et al*., 2014) [4].

The fruit peel wastes contain simple and complex sugars that are metabolizable by microorganisms (Saheed *et al*., 2013) [5] and have much attention for their conversion to bio-ethanol, biogas and animal feed (Tijani *et al*., 2012) [6]. Designing treatment schemes for specific agricultural residue limits efficiency of waste collection and prolong treatment period. Therefore, adoption of a method that accommodates several fruit wastes is highly robust, cheap and realistic in ameliorating impediments associated with fruit waste disposal (Aggelopoulos *et al*., 2014) [7]. The cultivation of microbial cells (bacteria, yeast, and fungi) that converts fruit wastes into value added products such as biomass that can serve as animal feed supplement is a unique approach.

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 of value added products has been reported which includes cellulase production by some fungi cultured on pineapple waste. Carotenoids production is carried out on agricultural waste using *Blakeslea trispora* (Papaioannou and Liakopoulou Kyriakides, 2012) [8] and cellulase enzyme production on agricultural waste by *Aspergillus niger*. Sugarcane bagasse has been also reported as an energy source for the production of lipase by *Aspergillus fumigatus* (Naqvi *et al*., 2013) [9].

The commercially available media are very costly. Routine practical require 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) [10].

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 *Penicillium chrysogenum* using fruit peel wastes as raw material.

Materials and Methods

Cleaning and Sterilization of glasswares

All the glasswares were first soaked in cleaning solution (100 g of potassium dichromate was added to 100 ml of distilled water followed by addition of 50 ml of concentrated sulphuric acid) for about 12 hrs and washed in tap water. Finally, they were cleaned with distilled water, dried and used for the study. All the media were sterilized in an autoclave at 15 lbs pressure for 20 minutes. The glasswares were sterilized at 160ºC for 1 hrs in Hot air oven.

Chemicals

All the chemicals used in the experiments were of analytical reagents (AR) grade and distilled water was used throughout the study.

Fruit Peels Collection

Fruit peels were obtained from freshly collected fruit such as Pine apple, Mango, Jack fruit, Green Banana, Yellow Banana, Sweet Lime and Pomegranate. The collected fruit peels were shade dried, powdered and stored at room temperature.

Isolation and identification of fungi from air

Fungal were isolated (*Aspergillus niger*, *Rhizopus stolonifer* and *Penicillium chrysogenum)* by Open plate technique. The Sabouraud's dextrose agar plates were prepared and opened in the Laboratory for 15 minutes. The plates were incubated at room temperature for 3 days.

Maintenance of fungal isolates

Well grown fungal colonies were maintained on Sabouraud's dextrose agar slants, stored at 4 °C and were identified by Lactophenol Cotton Blue (LPCB) staining and Plating on Sabouraud's Dextrose Agar methods

Inoculum preparation

The suspension of 4 days old cultures of fungi (*Aspergillus niger*, *Rhizopus stolonifer* and *Penicillium chrysogenum*) 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.

Qualitative growth analysis of fungi in fruit peel wastes

About 4.0 grams of fruit peel wastes were added into the 100 ml of distilled water and sterilized by autoclaving at 121°C for 15 minutes. After sterilization, the fruit peel waste broths were cooled and then one ml of industrially important fungal inoculums was added. The inoculum added broths were incubated at room temperature for 3 days. The presence/absence of the fungal growth was visually observed.

Results and Discussion

Table 1: Qualitative growth analysis of fungal in fruit peel wastes

S. No	Fruit peel wastes	*Aspergillus niger*	*Rhizopus stolonifer*	*Penicillium chrysogenum*
1	Pine apple	+	+	+
2	Mango	+	-	-
3	Jack fruit	+	-	-
4	Yellow Banana	-	-	-
5	Green Banana	+	+	+
6	Sweet Lime	-	+	+
7	Pomegranate	-	+	+

+ = Positive growth; - Nil Growth

Qualitative growth analysis of fungi in fruit peel wastes

The effect of seven different fruit peel wastes *viz*., Pine apple, Mango, Jack fruit, Yellow banana, Green banana, Sweet lime and Pomegranate on the qualitative growth of *Aspergillus niger, Rhizopus stolonifer and Penicillium chrysogenum* were studied and the results were given in Table 1. It was observed that the *Aspergillus niger* growth was recorded in the medium which contains Pine apple, Mango, Jack fruit and Green banana. The *Aspergillus niger* growth was not recorded in the medium containing Yellow banana, Sweet lime and Pomegranate (Fig 1). *Rhizopus stolonifer* growth was noticed in the medium which contains Pine apple, Mango, Sweet lime and Pomegranate. The *Rhizopus stolonifer* growth (Fig 2) was not recorded in the medium containing Yellow banana, Jack fruit and Mango. However, the *Penicillium chrysogenum* growth (Fig 3) was recorded in the medium which contains Pine apple, Mango, Sweet lime and Pomegranate. The *Penicillium chrysogenum* growth was not recorded in the medium containing Yellow banana, Jack fruit and Mango.

Agricultural waste materials supports 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) [11]. 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 (Prescot and Harley, 2002) [12]. 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, moisture (water) is required by all organisms for their life processes and fungi in particular require water for extracellular digestion of nutrients (Pelczar *et al*., 1993) [13], 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 (*A*spergillus *niger*, *Geotrichum candidum* and *Saccharomyces cerevisiae*) out of the four fungi. It thus produced the highest growth rates in these three

fungi. Oladiji *et al.* (2010) [14] reported that 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.

Fig 1: Growth analysis of *Aspergillus niger* in fruit peel extracts

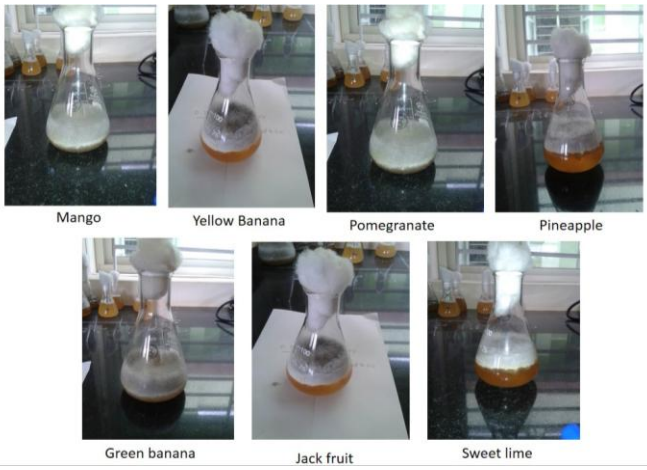

Fig 2: Growth analysis of *Rhizopus stolonifer* in fruit peel extracts

Fig 3: Growth analysis of *Penicillium chrysogenum* in fruit peel extracts

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 India. 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.

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