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RESEARCH PAPER

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Root crops based formulated culture media and its economic feasibility

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Abstract

The study aimed to determine which among the formulated fungal culture media in dehydrated form utilizing cassava, (*Manihot esculenta*), sweet potato (*Ipomoea batatas*), ube (*Dioscorea alata* L.), taro (*Colocasia esculenta*), and potato (*Solanum tuberosum*) shall best support the growth of fungi; and to determine the most effective level of the root crop utilized as fungal culture media. The fungus, *Saccharomyces cerevisiae*, *Aspergillus niger* and *Rhizopus stolonifer* isolates were used in the study. Inocula of *Rhizopus slotolonifer*, *accharomyces cerevisiae and Aspergillus niger were* taken asceptically and using a sterilized inoculating needle, each plate was inoculated in the center through stab technique. The size of the colony was measured through its diameter using a digital Vernier caliper and expressed in millimeters. Based on the findings of the study, cassava, sweet potato, ube, taro and potato with dextrose and agar powder showed comparable effects on the growth of fungi under the study. A level of 75%, 85% and 95% of cassava, sweet potato, ube, taro and potato dextrose agar supported the growth of *Saccharomyces cerevisiae*, *Aspergillus niger*, and *Rhizopus stolonifer*. A concentration of 75%, 85% and 95% of cassava, sweet potato rootcrop with dextrose and agar powder can be formulated into fungal culture media. The different formulations in this study are cost effective in the cultivation of fungi such as *Saccharomyces cerevisiae*, *Aspergillus niger* and *Rhizopus stolonifer*.

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Introduction

A substance that encourages the growth, support, and survival of microorganisms is known as microbiological culture medium. Culture media has been used by microbiologists since the nineteenth century. Even with the increased use of rapid methods the majority of techniques found in the pharmaceutical quality control laboratory require growth media. For the assessment of culture media, no one definitive standard exists. It contains nutrients, growth promoting factors, energy sources, buffer salts, minerals, metals, and gelling agents (for solid media). It is a mandatory requirement that culture media must contain high carbohydrate source, nitrogen source are required for the growth of fungi at pH range of 5 to 6, and a temperature range from 15 to 37°C. Since one of the standard approaches to the laboratory diagnosis of fungal infections is the cultivation of the causative fungus and its subsequent identification. For any fungus to be cultivated for any purpose, it is necessary to provide the appropriate biochemical and biophysical environments. The biochemical or nutritional environment is made available as culture medium (ASM, 2019).

However, these culture media are usually expensive and not readily available and thus their usage in small diagnostic laboratories has undoubtedly decreased. With this situation at hand, the protocols on the isolation and identification of the etiologic agent in a disease have been commonly addressed through growing the futative causal organism in artificial or natural media.

Thus this study was conceived utilizing simple culture media in dehydrated form using abundant naturally occurring resources such sweet potato (*Ipomoea batatas*), cassava, (*Manihot esculenta*), ube (*Dioscorea alata* L.), taro (*Colocasia esculenta*) and potato (*Solanum tuberosum*) were formulated to evaluate the growth of the fungi, *Saccharomyces cerevisiae, Aspergillus niger* and *Rhizopus stolonifer*.

Materials and methods

Research Design

Cassava (Manihot esculenta), Sweet potato (Ipomea batatas), Ube (Dioscorea alata L.), Taro (Colocasia

esculenta) and Potato (*Solanum tuberosum*) which were procured from local markets were used in the study. They were processed into powder, and the components of the culture media were the powdered root crop, dextrose and agar powder (food grade) mixed with distilled water. Proper amount of each component of the formulated culture media were prepared based on its formulation as follows: (75% has 18.0g root crop powder; 6.0g dextrose and 15g agar; 85% has 20.4g root crop powder; 3.6g dextrose and 15g agar; and 95% has 22.8g root crop powder; 1.2g dextrose and 15g agar).

The formulated culture media prepared was sterilized through an autoclave. Test fungi (*Saccharomyces cerevisiae*, *Aspergillus niger* and *Rhizopus stolonifer*), sterile inoculating needle, alcohol lamp, sterile petri dishes, and incubator were utilized in the cultivation of the fungi. A digital Vernier caliper, digital single lens reflex (DLSR) camera and a calculator were employed in data gathering. Commercial Potato Dextrose Agar was used as control.

Formulation of the Culture Media

There were five rootcrops used in the study namely: cassava (*Manihot esculenta*), sweet potato (*Ipomea batatas*), ube (*Dioscorea alata* L.), taro (*Colocasia esculenta*), and potato (*Solanum tuberosum*). Each rootcrop was peeled, washed, grated and oven-dried at 80°C. It was then pulverized and sieved. The root crop powder was combined with extrose powder, and agar powder (food grade) following the formulation in Table 1.

Preparation of the Formulated Culture Media

Using the formulations in Table 1, 39 grams of the formulated culture medium was suspended in 1000ml of distilled water. The agar powder acted as a gelling agent for the medium.

Preparation of the Potato Dextrose Agar

Thirty-nine (39) grams of Potato Dextrose Agar was suspended in 1000ml distilled water then boiled to dissolve the medium completely. The media was autoclaved for 15 minutes at 121°C and cooled to 45-50°C. The pH was adjusted then mixed well before dispensing on sterile plates.

The resulting solution was boiled until all constituents were dissolved. It was autoclaved for 15 minutes at 121°C. The pH was adjusted based on the following requirement of the fungi: *Saccharomyces cerevisiae* 4-6, *Aspergillus niger* 5.5 and *Rhizopus stolonifer* 7-8. The media was dispensed into sterile Petri dish, taking care to distribute equally at approximately 20-25ml per petri dish.

The Test Fungi

The fungus, *Saccharomyces cerevisiae*, used in the study was procured from the Regional Standards and Testing Laboratory of the Department of Science and Technology, Regional Office 1, DMMMSU-Mid La Union Campus, City of San Fernando, La Union while *Aspergillus niger* and *Rhizopus stolonifer* were local isolates.

Isolation of the Test Fungi, Aspergillus niger and Rhizopus stolonifer

An inoculum of *Rhizopus slotolonifer* was taken from moldy loaf bread using a sterilized inoculating needle. The cover of the prepared potato dextrose agar petri plate was gently raised and was stab inolucated at the center. The inoculated petri plate was incubated at a temperature of 25 oC for 5 days. After the incubation period, the fungus was identified through its morphological and cultural characteristics. Same procedure was carried out for *Aspergillus niger* but the source of the inoculum was a moldy garlic bulb.

Inoculation of Fungi

Each plate was inoculated in the center through stab technique for *Rhizopus stolonifer* and *Aspergillus niger*. For *Saccharomyces cerevisiae*, using a sterile inoculating needle an inoculum was taken from a stock culture and was inoculated at the center and to the sides of the plate. The inoculated plates were incubated at temperatures and periods required by the fungi which were as follows: *Saccharomyces cerevisiae* at 35°C for 3 days, *Aspergillus niger* at 25°C for 5 days and *Rhizopus stolonifer* at 25°C for 5 days days. After the incubation period, the colony size was measured.

Data Gathered and Analysis Colony size

The size of the colony was measured through its diameter using a digital Vernier caliper and expressed in millimeters. All data were expressed as mean + standard error of the mean (SEM). The gathered data were analyzed using standard deviation and Analysis of Variance (ANOVA). The Tukey's honestly significant difference (HSD) test and Post hoc test were used in the determination of the significance of the difference between means.

Results and discussion

Effective Level of the Root Crop Utilized as Fungal Culture Media

The growth of the fungi, *Saccharomyces cerevisiae*, *Aspergillus niger* and *Rhizopus stolonifer*, on the formulated culture media with different levels is shown in Table 1.

Table 1. Mean colony size of *Saccharomyces cerevisiae* on the different levels of the root crop on the formulated culture media (in mm).

Culture Media	Level (%)	Mean (mm)	
	75	5.15	
Cassava dextrose agar	85	5.07	
	95	5.03	
Sweet potate devtrose	75	5.30	
agar	85	5.20	
	95	5.20	
Ube dextrose agar	75	5.40	
	85	5.20	
	95	5.10	
	75	5.33	
Taro dextrose agar	85	5.10	
	95	5.13	
Potato dextrose agar	75	5.27	
	85	5.23	
	95	5.13	
Commercial PDA		5.87	

Growths of *Saccharomyces cerevisiae* are observed to be visible with the naked eye 5 hours post inoculation. *Saccharomyces cerevisiae* had a mean colony size of 5.17mm, 5.07mm, and 5.03mm on 75%, 85%, and 95% cassava dextrose agar respectively; 5.30mm, 5.20mm, and 5.20mm on 75%, 85%, and 95% sweet potato dextrose agar respectively; 5.40mm, 5.20mm, and 5.10mm on 75%, 85%, and 95% ube dextrose agar respectively; 5.33mm, 5.10mm, and 5.13mm on 75%, 85%, and 95% taro dextrose agar respectively and 5.27mm, 5.23mm, and 5.13mm on 75%, 85% and 95% cassava dextrose agar respectively. The commercial potato dextrose agar gave a colony size of 5.87mm for *Saccharomyces cerevisiae*. Analysis of variance (ANOVA) (Appendix Table 2b) revealed an insignificant difference among the level mean colony size grown in the formulated culture media. This signifies that 75 to 95% of the rootcrop on the formulated culture media can support the growth of *Saccharomyces cerevisiae*.

The results are in agreement with the studies by Ojocoh and Ekundayo (2005) on preparation of a medium from an infusion of sweet potato for culturing of yeasts from suitable suspensions of pure culture. Growth of yeast on the sweet potato agar (SPA) was comparable and sometimes higher than those obtained on potato dextrose agar (PDA).

The yeast species used as test organisms were Candida albicans, Geotrichum candidum. Rhodotorula sp., Saccharomyces cerevisiae Baker's yeast and Saccharomyces cerevisiae Brewer's yeast. Sweet potato agar was prepared from an infusion of sweet potato (Ipomoea batata) tubers. 10 grams of peeled tubers were cut into small pieces and boiled in 200ml distilled water until they became soft. After filtering the extract through muslin cloth, the filtrate was made up to 500ml with distilled water and agar (1.6% final concentration) was added to the filtrate. The filtrate was then divided into four batches, with each batch containing 100ml. The batches were supplemented separately with 0.3% yeast extracts, 0.5, 1.0 and 2.0% glucose before autoclaving. Each of the batches was further supplemented with a drop of lactic acid before pouring into plates as recommended (Ojocoh and Ekundayo, 2005).

Sweet Potato Sucrose Agar was indeed suitable for the cultivation of *Candida albicans*. The different concentrations of Sweet Potato Sucrose Agar exhibited appreciable growth in days that were comparable to the positive control group- Potato Dextrose Agar. Furthermore, as low as 200g of Sweet Potato infusion, it showed identical colony growth, with the same time

frame of growth in two days as that of Potato Dextrose Agar. The mean growth of *Candida albicans* using 200g and 400g Sweet Potato infusion was two (2) days. However, the 100g Sweet Potato infusion had a mean growth of four (4) days. Likewise, the mean growth of *Candida albicans* using the Potato Dextrose Agar was also two (2) days (Cale *et al.*, 2016).

Saccharomyces cerevisiae is reported as the most studied and biochemically best understood species of the yeast domain. It is best known for its domesticated role in the production of fermented products. This veast converts hexose sugars to ethanol, CO2, and a variety of compounds including alcohols, esters, aldehydes, and acids, that contribute to the sensory attributes of the food and beverage. Fermentation of carbohydrates in fruits, grains and other biomass to ethanol by S. cerevisiae is the critical process for a wide range of products from fine wines to gasoline additives. Glycerol is a sugar alcohol produced as a by-product of the ethanol fermentation process by Saccharomyces cerevisiae. It is an economically important alcohol with a slightly sweet taste and with applications in the food, beverage, pharmaceutical, and chemical industries. Glycerol can be produced by biochemical methods in which microorganisms are used (Yalcin and Yesim Ozbas, 2008).

Saccharomyces is yeast commonly isolated from human, mammals, birds, wine, beer, fruits, trees, plants, olives, and soil. Also known as the "baker's" or "brewer's" yeast, Saccharomyces cerevisiae is used in food industry in production of various food stuffs, wines, and beers. Saccharomyces cerevisiae is genetically tractable yeast which is closely related to Candida albicans. As a consequence, Saccharomyces cerevisiae is commonly used model yeast in fungal molecular research, including DNA sequence analysis, mechanism of action of and resistance to antifungal and the investigation of factors drugs, of pathogenicity, such as adhesion. Of note, Saccharomyces cerevisiae has also been used to express human granulocyte/macrophage colonystimulating factor (hGMCSF). While it is a common colonizer of mucosal surfaces and considered to be

nonpathogenic for immunocompetent hosts. Saccharomyces may cause infections particularly in immunocompromised patients (MSERC, 2021). Colonies of Saccharomyces grow rapidly and mature in 3 days. They are flat, smooth, moist, glistening or dull, and cream to tannish cream in color. The inability to utilize nitrate and ability to ferment various carbohydrates are typical characteristics of Saccharomyces. Blastoconidia are observed. They are unicellular, globose, and ellipsoid to elongate in shape. Multilateral (multipolar) budding is typical. Pseudohyphae, if present, are rudimentary. Hyphae are absent Saccharomyces produces ascospores when grown on V-8 medium, acetate ascospor agar, or Gorodkowa medium. These ascospores are globose and located in asci. Each ascus contains 1-4 ascospores. Asci do not rupture at maturity. Ascospores are stained with Kinyoun stain and ascospore stain. When stained with Gram stain, ascospores are gram-negative while vegetative cells are gram-positive (MSERC, 2021).

The effects of temperature, pH and sugar concentration (50% glucose+50% fructose) on the growth parameters of *Saccharomyces cerevisiae* T73, S. kudriavzevii IFO 1802T and the hybrid strain *S. cerevisiae* \times *S. kudriavzevii* W27 were studied by means of response surface methodology based in a central composite circumscribed design. Lag phase could not be properly modelled in the wine model system, where yeasts started the fermentation in few hours after inoculation. In the case of the maximum specific growth rate (µmax), the temperature was the most important variable for three yeasts, although the effects of sugar concentration (in T73 and W27) and pH (W27 and 1802) were also significant (pb0.05) (López *et al.*, 2009).

Another factor that should be considered with detail is the sugar concentration because yeast can delay its growth at high concentrations of glucose and fructose, which was clear for strains T73 and the hybrid W27. Sugar concentrations from 200g/L to 300g/L decreased *S. cerevisiae* growth rate. These authors found the lowest growth rate at the higher glucose concentrations. The response of *Saccharomyces* hybrids in laboratory media was studied under low pH (2.8) and 250g/L of glucose. The majority of yeasts tested were able to grow at 30 °C, but with the methodology used, they could not detect small changes in their responses. Therefore, the grape sugar concentration increase produced by the climatic change could negatively affect the kinetics of the wine fermentation (López *et al.*, 2009).

Andrietta et al. (2007) as cited Reis et al. (2014) reported that Saccharomyces cerevisiae remains the most utilised for ethanol production in Brazil. It is robust yeast that is capable of withstanding stressful conditions and has a high fermentation efficiency, rapid growth, effective sugar use, the ability to produce and consume ethanol, tolerance of high ethanol concentrations and low levels of oxygen, osmotolerance, thermotolerance, and cell activity in acidic environments, which are fundamental to its industrial usefulness. Stewart (2014) described that Saccharomyces cerevisiae is commercially significant in the food and beverage industries because of its role in the following: Production of fermented beverages and breads, Food spoilage, Processing food wastes, Source of food ingredients like Flavor compounds, δdecalatone, phenylethanol, yeast extract; Fractionated yeast cell componentsmannoproteins, glucomannans, yeast glycans, yeast protein concentrate, invertase, ergosterol, and glucans; Fructose syrup; and Probiotics (Saccharomyces boulardii). Cotoia (2020)concluded that Saccharomyces cerevisiae is a widely used model organism that has allowed scientists to better understand molecular, cellular, and biochemical processes, as well as the pathology and potential treatments to common human diseases.

The different levels of the formulated culture media supported the growth of *Aspergillus niger* in which hyphae were observed 7 hours post inoculation. The fungus has a mean colony size of 74.93mm, 71.80mm, and 71.65mm on 75%, 85%, and 95% cassava dextrose agar respectively; 74.47mm, 71.73mm, and 73.67mm on 75%, 85%, and 95% sweet potato dextrose agar respectively; 73.50mm, 72.80mm, and 71.27mm on

75%, 85%, and 95% ube dextrose agar respectively; 75.03mm, 73.33mm, and 71.13mm on 75%, 85%, and 95% taro dextrose agar respectively; and 74.83mm, 73.87mm, and 72.03mm on 75%, 85%, and 95% potato dextrose agar respectively. Its growth on the commercial Potato Dextrose Agar was noted to have a colony size of 87.57mm.

As shown in Appendix Table 3b, analysis of variance (ANOVA) revealed significant results on 85% and 95% of the root crops in the formulated media but they are significantly different from 75%. This means that the growth of *Aspergillus niger* is better when using 75% root crop. *Aspergillus niger* is the most common species of *Aspergillus*. It is known commonly to cause black mold in fruits and vegetables like grapes, apricot, onions, and peanuts. It is also known to cause food contaminations or food spoilages.

Table 2. Mean colony size of *Aspergillus niger* on the different levels of the root crop on the formulated culture media (in mm).

Culture Media	Level (%)	Mean (mm)
	75	74.93
Cassava dextrose agar	85	71.80
	95	71.63
Sweet potato dextrose agar	75	74.47
	85	71.73
	95	73.67
Ube dextrose agar	75	73.50
	85	72.80
	95	71.27
Taro dextrose agar	75	75.03
	85	73.33
	95	71.13
Potato dextrose agar	75	74.83
	85	73.87
	95	72.03
Commercial PDA		87.57

Aspergillus niger is highly thermotolerant therefore they can thrive in extreme temperatures including extremely low and extremely high conditions. Coupled with its asexual form of reproduction which makes it grown in any kind of environment when the conditions are favorable, and therefore it is also opportunistic. It ideally lives in decaying vegetation like compost piles and dead leaves, in soil and it can also be found in a lot of places including on grain stored with stored grains, dried fruits, dry nuts, and polyester (Mokobi, 2020). Macroscopic observation of *Aspergillus niger* reveals that their growth is initially white but they change to black after a few days producing conidial spore. Generally, they have a cottony appearance. The edges of the colonies appear pale yellow producing radial fissures. Macroscopic observation of colonies on potato dextrose agar at 25°C is initially white, which quickly becomes black with conidial production.

The reverse is pale yellow and growth may produce radial fissures in the agar. A microscopic view of *Aspergillus niger* reveals that *Aspergillus niger* has smooth colored conidiophores and conidia. The conidiophores are protrusions from septate and hyaline hyphae. The conidial heads appear radial and they split into columns (biseriate).

The conidiophore vesicle produces sterile cells known as metulae which support the phialides on the conidiophores. Beneficially, *Aspergillus niger* has been used for centuries in the production of citric acid that is a common food preservative in canned fruits, shampoos, and blood preservative. Other studies also revealed that it can be used in the production of glucoamylase, α -galactosidase, and many other industrially significant enzymes. It also produces glycoside hydrolase, an enzyme used to convert biomass into biofuels by breaking down the cellulose and hemicellulose from plant cell walls into a substance that can be used to produce bioactive metabolites, as well as other pharmaceutical products.

The growth of *Rhizopus stolonifer* on the different formulated culture media was evident at 7 hours post inoculation through the appearance of hyphae. It was observed after the incubation period that the colony of the fungus fully occupied the plate with a measurement of 89mm in all the formulated culture media and commercial PDA. As reported by Aryal (2020), colonies are fast-growing, covering the surface of the agar. Rapidly growing colonies fade from white to dark during sporulation. The colonies have a dense cottony growth or candy flossy or fairly floss in texture. Macroscopic observation of the color

of the colonies is initially white and turns grey to yellowish-brown as culture matures, Reverse pigmentation is white to pale.

Table 3. Mean colony size of *Rhizopus stolonifer* on the different levels of root crop on the formulated culture media (mm).

Culture media	Level (%)	Mean (mm)	
	75	89	
Cassava dextrose agar	85	89	
	95	89	
	75	89	
Sweet potato dextrose agar	85	89	
	95	89	
Ube dextrose agar	75	89	
	85	89	
	95	89	
	75	89	
Taro dextrose agar	85	89	
	95	89	
Potato dextrose agar	75	89	
	85	89	
	95	89	
Commercial PDA		89	

Rhizopus stolonifer is a threadlike mold and a heterotrophic species; it is dependent on sugar or starch for its source of carbon substances for food. It uses food matter, generally breads or soft fruits, like grapes or strawberries, as a food source for growth, nutrition and reproduction. R. stolonifer is a mass of mycelium, the vegetative filaments of the fungus, and a fruiting structure. Most of the mycelium is composed of multinucleate, rapidly growing hyphae. When the mold's spores are released they produce more mycelium through germination. As the mold matures it begins to turn black (Foody and Tong, 2008). As long as there is *R. stolonifer* spores in the air the mold can grow off of fruits, vegetables and bread products. The temperature and the location where the rhizoid starts to grow can have an effect upon the speed at which the mold grows. If there is limited air the spores will not be able to spread stopping R. stolonifer from growing altogether (Foody and Tong, 2008). R. stolonifer is an agent of plant disease; it breaks down organic matter through decomposition. When kept in a moist environment, such as a piece of bread, the parasite can quickly spread within a few days. Its spores are commonly found in the air.

The spores grow most rapidly at temperatures between 15°C and 30°C where they are able to germinate to their full potential. *Rhizopus* spp. causes infections in animals, humans, and plants. Human infection by *Rhizopus* spp. is known as mucormycosis also known as zygomycosis.

Plant diseases are collectively known as rots affecting harvested fruits (strawberries), tomatoes, sweet potatoes, tobacco, papayas, and stone fruits. It causes fruit rot on strawberry, tomato, and sweet potato (soft rot) (Foody and Tong, 2008; Aryal, 2020).

R. stolonifer has commercial use in the manufacturing of alcohol and organic acids. It is used in the commercial production of fumaric acid, lactic acid, and cortisone. R. arrhizus (R. oryzae) is useful for the production of lactic acid and cortisone, for alcoholic fermentation, and for the biosorption (passive adsorption of chemical contaminants by an organism) of heavy metals. R. delemar produces fumaric acid and biotin. In Asia several species are important in some foods, such as tempeh, and in many traditional alcoholic beverages (Foody and Tong, 2008; Aryal, 2020; Petruzzello, 2013).

Economic Feasibility

Table 4 presents the economic feasibility of using root crop culture media for the cultivation of fungi. It can be noted in the table that the cost of producing 500 grams cassava dextrose agar ranges from Php 256.44-801.32 while sweet potato dextrose agar ranges from Php 256.44-801.32, ube dextrose agar ranges from Php 262.29-805.94, taro dextrose agar ranges from Php 268.13-810.56 and potato dextrose agar ranges from Php 276.90-817.48. It can be noted that the cost of producing 500g of the different level of root crops in the formulated culture media are significant from each other.

Alternative culture media for fungal growth at low cost has been sought. Ravimannan *et al.* (2014) noted that the exorbitant costs of culture media have deprived the use of readymade culture media such as Potato Dextrose Agar (PDA) in schools and laboratories with financial limitations.

Generally legume seeds are found to be a good protein source for nutritional purposes. This study was carried out to find the feasibility of using legume seeds as an alternative nutrient source to grow fungi. Cowpea, green gram, black gram and soya meat (processed soya bean) were used in this study. The test organisms used were *Aspergillus, Trichoderma, Fusarium, Sclerotium* and *Pencillium* sp.

Table 4. Total cost of producing 500g of the formulated culture media at different levels (PhP).

Culture Media	75%	85%	95%
Cassava dextrose agar	801.32	528.89	256.44
Sweet potato dextrose agar	801.32	528.89	256.44
Ube dextrose agar	805.95	534.10	262.29
Taro dextrose agar	810.56	539.33	268.13
Potato dextrose agar	817.48	547.17	276.90
Commercial PDA		5573.62	

On average Fusarium sp. shows significantly (p<0.05) higher growth (5.85±0.18) in blackgram and Aspergillus shows significantly (p<0.05) less growth (1.58±0.31) in PDA. In comparison with the performance on conventional potato dextrose agar (PDA) media, the prepared protein formulations were found to be relatively cheap and good alternative culture media for mycological studies. Use of commercially available culture media for research purpose is costly so cheap culture media needs to be formulated. The feasibility of developing alternative culture media for PDA was assessed using locally vailable cheap materials such as Vegetables and fruits wastes, as they contain considerable amount of carbohydrate, protein and macro elements. These wastes are easily available in local shops, vegetable markets and kitchen. Waste such as Drumstick peel, seed; Cauliflower stalk, Potato peel, Fenugreek stem and orange peel was used to formulate media. Ability of media to support growth of Aspergillus and Trichoderma was tested. Growth on formulated media was comparable to commercially available media (Kadam et al., 2017).

Conclusion and recommendations

The study aimed to determine which among the formulated fungal culture media in dehydrated form utilizing cassava, (*Manihot esculenta*), sweet potato (*Ipomoea batatas*), ube (*Dioscorea alata* L.), taro

(*Colocasia esculenta*) and potato (*Solanum tuberosum*) shall best support the growth of fungi; to determine the most effective level of the root crop utilized as fungal culture media and its economic possibility.

The fungus, *Saccharomyces cerevisiae, Aspergillus niger* and *Rhizopus stolonifer* isolates were used in the study. An inoculum of *Rhizopus slotolonifer* was taken from moldy loaf bread using a sterilized inoculating needle. Each plate was inoculated in the center through stab technique for *Rhizopus stolonifer* and *Aspergillus niger*. The size of the colony was measured through its diameter using a digital Vernier caliper and expressed in millimeters. Based on the findings of the study, cassava, sweet potato, ube, taro and potato with dextrose and agar powder showed comparable effects on the growth of fungi under the study.

A level of 75%, 85% and 95% of cassava, sweet potato, ube, taro and potato dextrose agar supported the growth of *Saccharomyces cerevisiae, Aspergillus niger*, and *Rhizopus stolonifer*. A concentration of 75%, 85% and 95% of cassava, sweet potato, ube, taro, and potato rootcrop with dextrose and agar powder can be formulated into fungal culture media. Results showed that the formulated culture media can support the growth of fungi. A concentration of 75%, 85% and 95% 63 of cassava, sweet potato, ube, taro, and potato rootcrop with dextrose and agar powder can be formulated into fungal culture media. The different formulations in this study are cost effective in the cultivation of fungi such as *Saccharomyces cerevisiae*, *Aspergillus niger* and *Rhizopus stolonifer*.

References

Aryal S. 2020. Rhizopus spp-An overview. Retrieved on May 5, 2021 from https://microben otes.com/rhizopus-spp

ASM. 2019. American Society for Microbiology. Retrieved on December 7, 2020 from http://cmr. asm.org/cgi/content/full.

Basu S, Bose C, Ojha N, Das N, Das J, Pal M, Khurana S. 2015. Evolution of bacterial and fungal growth media. Bioinformation. **11(4)**, 182-184. Retrieved on December 6, 2019 from https://ncbi.nlm.nih. gov/pmc/articles/PMC4479053

Basu S, Pal A, Desai PK. 2005. Quality control of culture media in a microbiology laboratory. Indian Journal of Medical Microbiology **23(3)**, 159-163.

Cale CAG, Dela Peña RLS, Jumawan LV, Lapasa MRD, Frinxes G, Longakit GV, Mayol CGP, Omar AAI, Raffiñan AP, Simolde SM, Villamala KMS, Gernale JF. 2016. Sweet potato (*Ipomoea batatas*) sucrose agar as an alternative culture medium for *Candida albicans*. Retrieved on April 22, 2020 from http://www.herdin.ph /index.php?view=research&cid=68169#physiLoc

Foody E, Tong C. 2008. An Informative, Heart-Warming Tale About Black Bread Mold. Retrieved on May 4, 2021 f r o m http://tolweb. org/treehouses/? treehouse_id=4817.

Kadam A, Patil S, Sonne M, Dahigaonkar K, Oberoi JK, Jadhav J. 2017. Cost Effective Alternative Fungal Culture Media Formulation Using Fruit and Vegetables Waste. International Journal of Current Research **9(09)**, 56887-56893, September, 2017 ISSN: 0975-833X. Retrieved on December 6, 2019 from http://journalcra.com

López FN, Orlić S, Querol A, Barrio E. 2009. Effects of temperature, pH and sugar concentration on the growth parameters of *Saccharomyces cerevisiae, S. kudriavzevii* and their interspecific hybrid. International Journal of Food Microbiology **131**, 120-127. Retrieved on March 18, 2021 from www.elsevier. com/locate/ijfoodmicro

Microbiol. 2013 Published online 2014 Mar 10. Prepublished online 2014 Mar 4. DOI: 10.1590/S1517-83822014005000020. Dec; **44(4)**, 1121-1131. Retrieved on March 16, 2021 from https://ncbi.nlm. nih.gov/pmc/articles /PMC3958177 **Mokobi F.** 2020. *Aspergillus niger-* An overview. Retrieved on January 18, 2021 from https://microbenotes.com/aspergillus-niger

MSERC. 2021. Saccharomyces taxonomic classification. Mycoses Study Group. Education and Research Consortium. Retrieved on March 16, 2021 from https://drfungus.org/knowledge-base/sacchar omyces-specie

Petruzzello M. 2013. Rhizopus. Encyclopædia Britannica, Inc. Retrieved on April 24, 2021 from https://www.britannica.com/science/Rhizopus.

Reis VR, Bassi APG, da Silva JCG, Ceccato-Antonini SR. 2014. Characteristics of *Saccharomyces cerevisiae* yeasts exhibiting rough colonies and pseudohyphal morphology with respect to alcoholic fermentation. Braz J.

Stewart GC. 2014. Saccharomyces, *Saccharomyces cerevisiae*. Encyclopedia of Food Microbiology (Second Edition), Retrieved on May 11, 2021 from https://www.sciencedirect.com/topics/neuroscience /saccharomyces-cerevisiae

Yalcin SK, Yesim Ozbas Z. 2008. Effects of pH and Temperature on Growth and Glycerol Production Kinetics of Two Indigenous Wine Strains of *Saccharomyces cerevisiae* from Turkey. Brazilian Journal of Microbiology **39**, 325-332 ISSN 1517-8382