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Full Length Research Paper

Utilization of food crop wastes for the formulation of laboratory media used for cultivating soil fungi.

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A major problem experienced by both developed and developing countries is the management of wastes. Thus, there is a growing interest regarding the conversion of organic wastes generated by the food processing sector and through other human endeavors into useful forms. An investigation was carried out to test the suitability of food crop wastes (yam, sweet potato, carrot and Irish potato peels) for the formulation of media for cultivating soil fungi. Four formulated media (Yam Peel Dextrose agar (YPDA), Sweet Potato Peel Dextrose Agar (SPPDA), Carrot Peel Dextrose Agar (CPDA), Irish Potato Peel Dextrose Agar (IPPDA) and Sabouraud Dextrose Agar (SDA) were used for the isolation of soil Fungi. Mean radial growths of the isolates on the formulated waste media were determined and compared with that of SDA which served as the control. The test organisms were aseptically inoculated unto the four different formulated media and the control medium in triplicates. The cultures were incubated for a period of six days and the radial growth of each of the fungi was measured thereafter. Growth rates on the test media were also calculated for each fungus. The fungal isolates from this study were *Aspergillus niger, Geotrichum candidum, Aspergillus flavus* and *Saccharomyces cerevisiae.* All the formulated media supported the growth of the test fungi at various degrees. There were significant differences (P<0.05) in the mean radial growths (MRG) of all the test fungi on the different media. Sweet Potato Peel Dextrose Agar (SPPDA) gave the highest MRG of 8.10 cm, 8.10cm and 7.40cm for *A. niger*, *G. candidum* and *S. cerevisiae* respectively. The MRGs of these three fungi on SPPDA were found to be higher than their MRGs on all the other media including the control medium (SDA). The control SDA yielded the highest MRG for *A. flavus*. Yam Peel Dextrose Agar (YPDA) yielded the lowest MRG values for all the test fungi. Seventy-five percent of the fungi demonstrated higher growth rates on SPPDA. There is promise for the use of SPPDA for the cultivation of the test soil fungi.

KEY WORDS: Food crop, wastes, fungal isolates, laboratory media, radial growth

INTRODUCTION

Wastes are materials that have not yet been fully utilized. They are leftovers from production and consumption.

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However, waste is an expensive and sometimes unavoidable result of human activity. It includes plant materials, agricultural, household, industrial and municipal wastes and residues (Okonkwo *et al*., 2006). The disposal of agricultural wastes on land and into water bodies is common and has been of serious ecological hazards (Smith *et al*., 1987). In developing countries,

there is a growing interest regarding the utilization of organic wastes generated by the food processing sector and through other human endeavors. This has led to a new policy geared towards complete utilization of raw materials so that little or no residue is left to pose pollution problems (Ofuya and Nwajuiba, 1990). Insufficient and improper methods of disposal of solid wastes result in scenic blights, serious hazards to public health (including pollutions of air and water resources), accident hazards and increase in rodents and insect vectors of disease. Improperly handled wastes ultimately become a nuisance to the public and interfere with community life and development (Tchobanoglous and Theisen, 199). The agricultural-based industries generate significant quantities of organic wastes including peels from cassava, plantain, banana, oranges and straw from cereals. Rather than allow these wastes to become solid municipal wastes, it is necessary to convert them to useful end products. It is now realized that these waste could be utilized as cheap raw materials for some industries or as cheap substrates for microbiological processes (Nwabueze and Otowa, 2006). The food processing industry generates a large amount of wastes annually including crop residues like peels, husks, cobs, and shells (Gomez-Pazos *et al*., 2005). Such wastes are rich in sugar and are easily assimilated by microorganisms; this makes the wastes suitable materials for growth of microorganisms. Inability to salvage and reuse such materials economically results in the unnecessary waste and depletion of natural resources (Selke, 1990; Tchobanoglous and Theisen, 1993).

Fungi constitute one of the largest groups of plants with richest arrays of species. They are a group of eukaryotic spore bearing, achlorophyllous organisms that generally reproduce asexually and sexually (Pelczar *et al*., 1993). Some are agents of diseases in plants (parasitic), while others are saprophytic. Saprophytic fungi tend to be responsible for most of the disintegration of organic materials, and some of them render food material toxic (Pelczar *et al*., 1993). The saprophytic fungi represent the largest proportion of fungal species and they perform a crucial role in the decomposition of plant chemical compounds such as cellulose, hemi-cellulose and lignin, thus, contributing to the maintenance of the global carbon cycle. Fungi grow on diverse habitat in nature and are cosmopolitan, requiring several specific elements for growth and reproduction. In the laboratory, fungi are isolated on specific culture media for cultivation, preservation, macroscopic examination and biochemical and physiological characterization. A wide range of media are used for isolation of different groups of fungi. These media influence vegetative growth, and colony morphology, pigmentation and sporulation depending on their composition, pH, temperature, light, water availability and surrounding atmospheric gas mixture (Northolt and Bullerman, 1982; Kuhn and Ghonnoum, 2003).

Fungi are an important component of soil microbiota typically having higher biomass than bacteria depending on soil depth and nutrient conditions (Ainsworth and Bisby, 1995). 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 [8]. 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). The feasibility of developing alternative media for cultivation of fungi apart from the conventional ones like Sabouraud Dextrose Agar (SDA) and Potato Dextrose Agar (PDA) has been studied by different researchers (Weststeijn and Okafor, 1971; Adesemoye and Adedire, 2005; Tharmilla and Thavaranjit, 2011). The need to develop alternative media has become imperative as the conventional media are either not readily available or expensive in most developing countries like Nigeria (Weststeijn and Okafor, 1971). The aim of this study was to exploit the suitability of food crop wastes such as yam, sweet potato, Irish potato and carrot peels in the formulation of laboratory media used for the cultivation of soil fungi.

MATERIALS AND METHODS

Sample Collection and Preparation

Wastes (peels) obtained by cutting of the outer surface of Yam, Irish potato, Sweet potato and Carrot were collected from some houses in Jos, Plateau State, Nigeria. The wastes were sorted out and each type of waste material was washed, sundried and then milled into powdery form. The milled peels were stored in sterile plastic airtight containers. The test organisms used in the experiment were isolated from soil samples collected from three different gardens in Jos. Twenty grammes of the soil was collected randomly in replicates of five from each location using a sterile spatula. The samples were collected from the top 2cm soil in the garden into sterile polythene bags and taken to the laboratory. The test organisms were isolated from the soil samples using Sabouraud Dextrose Agar (SDA). One gramme of soil sample was weighed into a sterile tube containing 9ml of sterile distilled water to produce a stock suspension (10^{-1}). The stock suspension was shaken vigorously for 5 seconds to dislodge the soil organisms. After this, serial ten-fold dilution was carried out up to 10^{-7} dilution. Using

Table 1: Nutrient Composition of Crop Wastes (Yam Peel, Sweet Potato Peel, Carrot Peel and Irish Potato Peel)

Crop waste	CB (mg/100g)	CP (mg/100g)	AS (mg/100g)	CF (mg/100g)	MO (mg/100g)	Ca (mg/kg)	Na (mg/kg)	Mg (mg/kg)	P (mg/kg)
YP	22.20	1.50	1.30	0.17	0.06	12.00	0.86	0.72	2.30
SPP	20.10	1.60	2.37	3.90	0.06	30.00	5.50	25.10	37.20
CP	0 7.60	1.00	2.90	3.70	0. 05	29.10	2.40	19.00	24.00
IPP	14.00	1.76	1.45	2.90	0.06	12.00	6.00	23.00	05.80

YP = Yam Peel, SPP = Sweet Potato Peel, CP = Carrot Peel, IPP = Irish Potato Peel, CB = Carbohydrate, CP = Crude protein, AS = Ash content, CF = Crude fibre content, MO = moisture content, Ca = Calcium, NA = Sodium, Mg = Magnesium, P = Potassium

the pour plate method the cultures were prepared and incubated at 27^{O}C for 5 days. Pure cultures of the various fungi that grew on the medium were prepared by sub-culturing and kept for microbiological examination.

Chemical Analysis

The chemical compositions (carbohydrate, crude protein, crude fibre and ash) of the wastes (yam, sweet potato, carrot and Irish potato peels) were determined according to the method described by Ruth *et al*. (2012), while mineral were analyzed using Atomic Absorption spectrophotometer (AAS).

Characterization and Identification of Fungal Cultures

A small amount of aerial growth of each fungus was removed using mounted needles and transferred to a drop of cotton blue in lactophenol on a clean slide. The hyphae were teased apart with the needles and a cover slip was placed over the preparation taking care not to trap air bubbles. The preparation was viewed under the microscope. The nature of hyphae and fruiting structures were useful in the identification of the fungi. The x100 oil immersion objective was used for observation of details of spore attachment, surface texture, ornamentation of hyphae and spores. After the process of characterization the fungal isolates were identified by making references to (Barnett *et al*., 1983) and (Domsch and Anderson, 1980).

Media Formulation and Determination of Fungal Radial Growth.

Four different media were formulated, namely Sweet Potato Peel Dextrose agar (SPPDA), Irish Potato Peel Dextrose Agar (IPPDA), Yam Peel Dextrose Agar (YPDA) and Carrot Peel Dextrose Agar (CPDA). Five grammes of each milled waste was introduced into separate 500ml conical flasks. Twenty grammes of glucose and 7.5g of agar were also added. Distilled water was added to make each medium up to 500ml. The medium was shaken vigorously so as to obtain a homogenous mixture. After autoclaving, each of the media was allowed to cool to about 45^{O}C before adding 1ml of 0.005g of chloramphenicol (this was added to inhibit bacterial growth). For comparative analysis, a conventional mycological media SDA was also prepared according to manufacturers (*Oxoid*) specification, and used as control. The suitability of the formulated media was estimated by culturing the test fungi on them. With the aid of a sterile cork borer a 5mm mycelial disc of each fungus was obtained from a 5-day old culture of the fungus and used to centrally inoculate triplicate plates of the formulated media and the control SDA medium. The plates were incubated at 27^{O}C for 6 days and the radial growth of each fungus on the various media was measured and recorded at the end of the incubation period. The mean radial growths of the fungi on the formulated media were compared with their mean radial growths on SDA which was the control. Growth rates of the test fungi on the different media were also calculated from the results of the mean radial growths.

Statistical Analysis

Analysis of variance was used for the statistical analysis of the results. LSD was used to separate means with statistically significant differences.

RESULTS

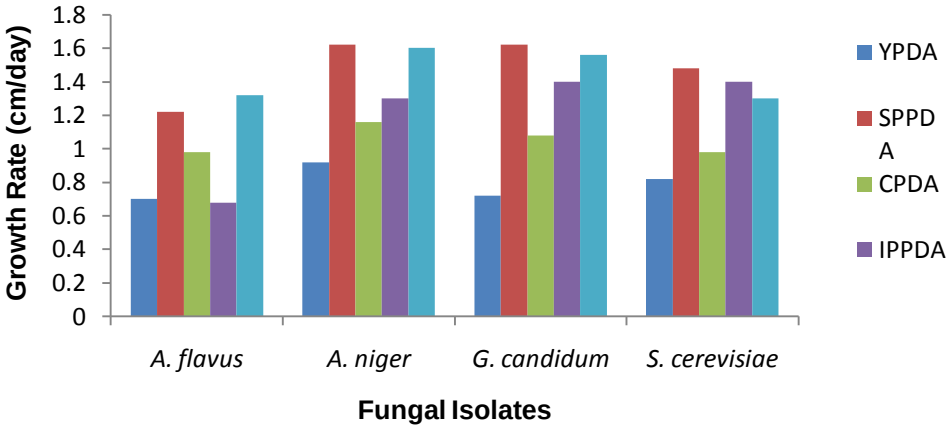
The nutrient contents of the different waste materials are shown in Table 1. The fungal isolates from this study were *Aspergillus niger, Geotrichum candidum, Aspergillus flavus* and *Saccharomyces cerevisiae.* All the formulated media supported the growth of the test fungi at various degrees. There were significant differences ($p<0.05$) in size of radial growth produced on the different media for all the test fungi. Sweet Potato Peel Dextrose Agar (SPPDA) gave the highest mean radial growths (MRG) of 8.10 cm, 8.10cm and 7.40cm for *A. niger*, *G. candidum* and *S. cerevisiae* respectively. The MRGs of

Table 2: Mean Radial Growth of the Test Fungi on the Formulated Media and on Sabouraud Dextrose Agar

Medium	Mean Radial Growth (cm)			
	A. flavus	*A. niger*	*G. candidum*	*S. cerevisiae*
YPDA	3.50 ± 0.05^{a}	4.60 ± 0.05^{a}	3.60 ± 0.00^{a}	4.10 ± 0.10^{a}
SPPDA	6.10 ± 0.10^{c}	8.10 ± 0.00^{d}	8.10 ± 0.10^{e}	7.40 ± 0.09^{e}
CPDA	4.90 ± 0.13^{b}	5.80 ± 0.01^{b}	5.40 ± 0.10^{b}	4.90 ± 0.20^{b}
IPPDA	3.40 ± 0.05^{a}	6.50 ± 0.09^{c}	7.00 ± 0.05^{c}	7.00 ± 0.20^{d}
SDA	6.60 ± 0.09^{c}	8.00 ± 0.05^{d}	7.80 ± 0.20^{d}	6.50 ± 0.05^{c}

YPDA = Yam Peel Dextrose Agar, SPPDA = Sweet Potato Peel Dextrose Agar, CPDA = Carrot Peel Dextrose Agar, IPPDA = Irish Potato Peel Dextrose Agar, SDA = Sabouraud Dextrose Agar

Fig 1: Growth Rates of the Test Fungi on the Formulated Media and Sabouraud Agar



YPDA = Yam Peel Dextrose Agar, SPPDA = Sweet Potato Peel Dextrose Agar, CPDA = Carrot Peel Dextrose Agar, IPPDA = Irish Potato Peel Dextrose Agar, SDA = Sabouraud Dextrose Agar

these three fungi on SPPDA were found to be higher than their MRGs on all the other media including the control medium (SDA). On SPPDA, *G. candidum* and *S. cerevisiae* had significantly higher (P<0.05) MRG values than when they were grown on the control SDA. The difference in the MRGs of *A. niger* on SPPDA and on the control medium were, however, not significant. The control SDA yielded the highest MRG for *A. flavus*. It was closely followed by SPPDA. Though higher, the MRG of the control medium was not significantly higher than that of SPPDA. Yam Peel Dextrose Agar (YPDA) yielded the lowest MRG values for all the test fungi. Details of mean radial growths of the fungi on the various media are presented in Table 2. Fungal growth rates were found to be higher on SPPDA for all the fungi except for *A. flavus* in which case growth rate was highest on the control SDA. Details of growth rates are given in Figure 3.

DISCUSSION

Results of the study revealed that all the formulated media supported the growth and sporulation of all the test fungi, though, at varying degrees. This is in conformity with the findings of (Weststeijn and Okafor, 1971; Adesemoye and Adedire, 2005; Tharmilla and Thavaranjit, 2011; Ruth *et al*., 2012) who reported the use of alternative culture media for growing fungi. The growth of the fungi on the formulated media implies that the wastes (peels) which were used in formulating the

media contained the required nutrients for fungal growth (Table 1). Microbiological studies depend on the ability to grow and maintain microorganisms under laboratory conditions by providing suitable culture media that offer favourable conditions (Domsch and Anderson, 1980; Beever and Bollard, 1970). 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). 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. In terms of mean radial growth, Sweet Potato Peel Agar (SPPDA) was found to be the best media for growing three (*A*. *niger*, *G*. *candidum* and *S*. *cerevisiae*) out of the four fungi. It thus produced the highest growth rates in these three fungi. In conformity with the present finding, (Amadi and Moneke, 2012) also reported higher mycelia growth rate in purple sweet potato dextrose agar than in yam dextrose agar. The ability of SPPDA to support good growth of the fungi shows that it not only contained the right nutrients but also probably contained them in the right proportions. The fact that the fungi grown on SPPDA performed better in most cases than when they were grown on the conventional SDA shows that SPPDA could serve as a good and possibly cheaper alternative medium for the cultivation of some soil fungi.

CONCLUSION

The study has revealed that the waste materials contain minerals and nutrients that can meet the nutritional requirements of fungi, thus they can be utilized as an alternative materials in the formulation of culture media for the *in vitro* cultivation of fungi for teaching and research purposes. An important advantage of the food crop peels used in formulating the various media is that it readily available in Plateau State, Nigeria (where the research was carried out). 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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