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Microbial Media Formulation Using Rice Husks, Yam and Cassava Peels on Some Selected Microorganisms

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Abstract: A research was carried out to find the feasibility of food crop waste (cassava peel, rice husk and yam peel) for formulation of media for cultivating fungi. Three formulated media which included Cassava Peel Extract Agar (CPEA), Rice Husk Extract Agar (RHEA) and Yam Peel Extract Agar (YPEA). Also, three Fish Extract Enriched formulated media which included Enriched Cassava Peel Extract Agar (ECPEA), Enriched Rice Husk Extract Agar (ERHEA) and Enriched Yam Peel Extract Agar (EYPEA) were used to determine the growth of two test fungi isolated from yam rot and harp beer respectively. The test organisms were aseptically transferred using ethanol sterilized cork borer of 0.8 cm to the formulated media and control medium. The colony diameter of the test fungi on the different media was measured after every two days for 10 days. A graph of colony diameter (cm) was plotted against time (days). The growth characteristics which included lag phase, specific growth rate and mean generation time of the test organisms were determined in triplicates. Potato dextrose agar served as control. The fungal isolates were *Saccharomyces carlsbergensis* and *Penicillium species.* All formulated media supported the growth of test organisms at various degrees. *Saccharomyces carlsbergensis* on formulated media had highest lag phase at 1.30days and 1.33 days, specific growth rate in YPEA at 0.97day and 0.77day, then mean generation time in CPEA at 0.83day and 1.98 days respectively. *Penicillium species* had the highest lag phase on CPEA 1.4days, specific growth rate on RHEA at 0.95 days and mean generation time on YPEA at 1.33days. On enriched media, lag phase was highest on YPEA at 1.50days, specific growth rate was on both RHEA and YPEA at 0.77 days. Then, mean generation time was highest on CPEA at 1.98days. The proximate composition of the extracts were determined with the aid of Association of Official Analytical Chemists (AOAC). The results were subjected to analysis of variance (ANOVA) (*P ? 0.05*). The result revealed that there was a significance difference in the protein, carbohydrate, vitamin C and mineral contents of the extracts analyzed. The alternative media formulated using cassava peel extract, rice husk extract and yam peel extract as substrate was found to be good for isolation and cultivation of microbes.

Key words: Yampeel extract · Cassava peelextract · Rice husk extract

INTRODUCTION

Media used in the laboratory for the cultivation of microorganisms supply the nutrients required for microbial cellular growth and maintenance. A wide variety of culture medium is employed by the microbiologist for the isolation, growth, maintenance of pure cultures and identification of microorganisms according to their biochemical and physiological properties [1].

Yam peels are low in crude fiber and rich in mineral matter, carbohydrates and certain amounts of vitamins [2]. The tuber peels are also richer in ash, fat, protein and crude fiber than the tissue of the tubers [2].

Cassava peels are lignocellulosic materials which consist of three main components, namely, cellulose, hemicellulose and lignin. Being very abundant, cassava peels can be use to formulate media for microbial growth.

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The dry outer covering of rice grain is known as rice husk, it is always removed during the milling of rice and of no direct nutritional value to man. In some areas however, it may be collected and used as litter material or used in fire making or allowed to rot away. Since rice offal makes up to 40% of parboiled rice. Also, rice husk is one of the most commonly available lignocellulosic materials that can be converted to different types of fuels and chemical feed stocks through a variety of thermochemical conversion processes [3].

A substantial amount of wastes is generated annually from food processing industry including crop residues like peels and husks [4]. The disposal of these agricultural wastes generated by the food processing sector and through other human endeavors into the environment (land and water bodies) has been of serious ecological hazards [5]. The peels (waste) from cassava, yam and the husk from rice, when not properly disposed, results in serious hazard to public health, also pollutions of air and water resources leading to disease outbreak, increase in rodents, insects vectors of diseases and accident hazards [6]. Proper and complete utilization of these wastes minimize the risk of pollution as little or no residue will be left [7].

Also, the increasing cost of conventional culture media in local market of developing nations like Nigeria, has necessitated continuous search for more readily available culture media at affordable prices [8]. The media in most cases are not readily available. Although the substances used in the production of culture media are normally in the form readily absorbed by the microorganisms, complex organic substances derived from natural agricultural waste including rice husk, yam peels and cassava peels are used as substrates for microbial growth [9]. To develop such important microbiological media, we have to search, evaluate, formulate and optimize media from these waste from non-conventional plant sources, such type of wastes may be served as alternative to substitute these expensive media produced from different companies [10].

This study was aimed at formulating culture media for the isolation of microorganisms using locally available substances which are disposed off as domestic wastes such as yam peels, cassava peels and rice husk.

MATERIALS AND METHODS

Collection of Samples: Approximately 45kilograms each of agricultural wastes including cassava peel, rice husk and yam peels were obtained from.

Evaluation of Nutritional Composition: The nutritional composition of the samples (cassava peel, rice husk and yam peel) were evaluated using different methods. Crude protein was determined using the micro-kjeldhal method, vitamin C by visual titration method and total carbohydrate by Anthrone method (AOAC, 2010).

Mineral Analysis: The mineral composition of cassava peel extract, rice husk extract and yam peel extract included calcium, magnesium, manganese, iron, zinc and sodium were analyzed using AOAC [11].

Test Organisms: The test organisms used were, *Saccharomyces carlsbergensis* and *Penicillium species* were locally isolated from harp beer and yam rots respectively. A pure culture of the microorganisms was maintained on a slant Potato dextrose agar (PDA) and was sub cultured on Potato dextrose agar plate. It was incubated aerobically for 3 to 5 days at room temperature.

Media Formulation: The agricultural wastes collected including cassava peel, rice husk and yam peel were sundried for about 4 to 5 days and blended into powdery form. The samples were stored separately in a clean airtight polythene bag.

Liquid Media Formulation: Three different broth (CPB: cassava peel broth, RHB: rice husk broth and YPB: yam peel broth) were prepared as follows; approximately, 65grams each of the ground samples (yam peel, cassava peel) and rice husk were weighed separately into measuring cylinder and volume made up to two liters (2L) with distilled water. The mixture was transferred carefully into a sterile plastic bucket (10 liters) and allowed to soak for 24 hours. This allowed for fermentation of the agricultural waste samples to take place. The filtrate was decanted using a muslin cloth whereas the residue was discarded. The filtrate was refiltered using a filter paper to remove tiny particles of the waste samples. The filtrate was tyndalized by heating in a water bath for 60 minutes at 98°C to stop the fermentation process. The broth was finally sterilized at 121°C for 15minutes and stored in a refrigerator when not in use.

Solid Media Formulation: Cassava peel extract agar (CPEA), Rice husk extract agar (RHEA) and Yam peel extract agar (YPEA) were formulated. Approximately 15grams of agar (Oxoid) which is a solidifying agent was weighed and dissolved into one liter (1L) of each

formulated liquid medium. The solid media were sterilized at 121ºC for 15 minutes and approximately 20 ml of the sterilized medium was distributed into each of the sterile Petri dishes prior to inoculation.

Enrichment of Formulated Media: Three different enriched formulated media was prepared which included Enriched Cassava Peel Extract Agar (ECPEA), Enriched Rice Husk Agar (ERHEA) and Enriched Yam Peel Extract Agar (EYPEA). Fish extract was used to enrich the media. The fish sample was collected from Nwakpu market the local market at Ikwo. After proper washing, the fish was soaked in a sterile water for 24hours. The extract was then steamed for 5 minutes at 98ºC using a water bath. 1ml of the extract was aseptically injected into a sterile petri dishes using a sterile syringe, then 20ml of sterilized formulated medium was added. The mixture was swirled a little and then allowed to gel.

Microbial Inoculation: *Saccharomyces carlsbergensis* and *Penicillium species* were inoculated unto the different formulated media which included cassava peel extract agar (CPEA), rice husk extract agar (RHEA), yam peel extract agar (YPEA) and the enriched media, enriched cassava peel extract agar (ECPEA), enriched rice husk agar (ERHEA) and enriched yam peel extract agar (EYPEA). A cork borer of about 0.8cm was used in inoculation. An ethanol sterilized cork-borer was passed through flame to remove the ethanol prior to cutting the circular disks of *Saccharomyces carlsbergensis* and *Penicillium species* culture from the perimeter portion of the colony. This region of the colony represents the youngest growth and so was used as a standardized inoculum. Using the sterilized cork-borer, a circular disk was cut on the agar plates of the formulated media, CPEA, RHEA, YPEA and ECPEA, ERHEA and EYPEA respectively of which the agar was discarded. Another circular disk was also cut on a pure culture agar plate of *Saccharomyces carlsbergensis* and *Penicillium species*, then, using a sterilized wire loop, one disc each containing *Saccharomyces carlsbergensis* and *Penicillium species* were aseptically transferred onto the center of each of the formulated agar plates (CPEA, RHEA and YPEA) respectively taking care not to cause any further damage to the organisms. The disk was inverted so that the organisms comes in contact with the media. The plates were aerobically incubated at room temperature for 3-5 days. The growth diameter of *Saccharomyces carlsbergensis* and *Penicillium species*were measured using a meter rule after every two days for a period of nine days (9 days), then the lag phase period, specific growth rate and mean generation time (doubling time) of the organisms on different formulated media and enriched media was determined. For comparative analysis, a conventional mycological media PDA was prepared according to manufacturers (Oxoid) specification and used as control.

Statistical Analysis: Three replicates of the results were statistically analyzed per sample by SPSS software (version 16.0) and the data generated was subjected to analysis of variance, the means were compared using the Duncan Multiple range test (DMRT). The level of statistical significance was set at $p<0.05$.

RESULTS

The mineral composition of the cassava peel extract, Rice husk extract and yam peel extract (Table 1) can be comparable to the composition of potato dextrose agar. The growth curves of Saccharomyces carlsbergensis and Penicillium species on the different formulated media were comparable to the growth curves obtained for potato dextrose agar (Figures 1 – 8), (tables 2 - 4). Also, when compared, there was no statistical difference in the pattern of growth, growth characteristics and colonial morphologies between the formulated media and potato dextrose agar which was used as the standard media for fungal isolation.

When enriched with fish extract, the media did not show better results in supporting the growth of the test organisms when compared with the formulated media hence the result showed that the media needs not be enriched to support the growth and isolation of fungi as it contained the basic requirements to support fungal growth. This study has shown that other locally available substances which are currently disposed off as agricultural wastes can be put to better use for the isolation of fungi.

The population density of *Saccharomyces carlsbergensis* on dilutions 10^{-1}, 10^{-3}, 10^{-5}, 10^{-7} and 10^{-9} was 5.2×10^{3} cfu/ml, 4.3×10^{5} cfu/ml, 3.9×10^{7} cfu/ml, 2.5×10^{9} cfu/ml respectively. Also, the population density at different dilutions, 10^{-1}, 10^{-3}, 10^{-5} and 10^{-7} of *Penicillium specie* obtained from yam rot was 4×10^{3} cfu/g, 3.4×10^{5} cfu/g, 2.9×10^{7} cfu/g and 1.5×10^{9} cfu/g respectively.

Table 1: Proximate and Mineral Compositions of Cassava Peel Extract, Rice Husk Extract and Yam Peel Extract.

	Cassava Peel Extract	Rice Husk Extract	Yam Peel Extract
Crude Protein	$0.3060^{a} \pm 0.0020$	$0.3307^{b} \pm 0.0081$	$0.4427^{c} \pm 0.0151$
Carbohydrate	$0.0213^{b} \pm 0.0061$	$0.0160^{a} \pm 0.0020$	$0.0127^{a} \pm 0.0012$
Vitamin C	$4.8660^{b} \pm 0.0225$	$4.0773^{a} \pm 0.0197$	$7.3223^{c} \pm 0.0254$
Calcium	$0.1920^{a} \pm 0.0020$	$0.2447^{a} \pm 0.0479$	$0.1907^{a} \pm 0.0083$
Manganese	$0.0330^{a} \pm 0.0020$	$0.1640^{b} \pm 0.0020$	$0.1640^{b} \pm 0.0020$
Zinc	$0.0370^{b} \pm 0.0020$	$0.0290^{a} \pm 0.0020$	$0.0290^{a} \pm 0.0020$
Sodium	$5.0200^{a} \pm 0.0200$	$12.9067^{c} \pm 0.0491$	$12.9067^{c} \pm 0.0491$
Magnesium	$5.5993^{c} \pm 0.0090$	$5.2130^{b} \pm 0.0305$	$4.5207^{a} \pm 0.0439$
Iron	$0.0460^{a} \pm 0.0020$	$0.1390^{c} \pm 0.0020$	$0.0930^{b} \pm 0.0020$

Table 2:The Growth Characteristics of *Penicillium species* and *Saccharomyces carlsbergensis* on Potato Dextrose Agar (PDA).

Test Organisms	Lag phase (Days)	Specific growth rate (Per/day)	Mean generation time (Days)
Saccharomyces carlsbergensis	1.00	0.85	0.82
Penicillium species	1.00	0.83	0.83

Table 3: The growth characteristics of *Saccharomyces carlsbergensis* on formulated media.

	Lag Phase (Days)	Specific Growth Rate (Per/day)	Mean Generation Time (Days)
Cassava Peels Extract Agar (CPEA)	1.30	0.83	0.83
Rice Husk Extract Agar (RHEA)	2.20	0.92	0.75
Yam Peels Extract Agar (YPEA)	0.83	0.97	0.71

Table 4: The Growth Characteristics of *Saccharomyces carlsbergensis* on the Formulated Media Enriched with Fish Extract.

	Lag Phase (Days)	Specific Growth Rate (per/day)	Mean Generation Time (Days)
Cassava Peels Extract Agar (ECPEA)	1.00	0.33	2.10
Rice Husk Extract Agar (ERHEA)	2.10	0.71	0.98
Yam Peels Extract Agar (EYPEA)	1.60	0.77	0.90

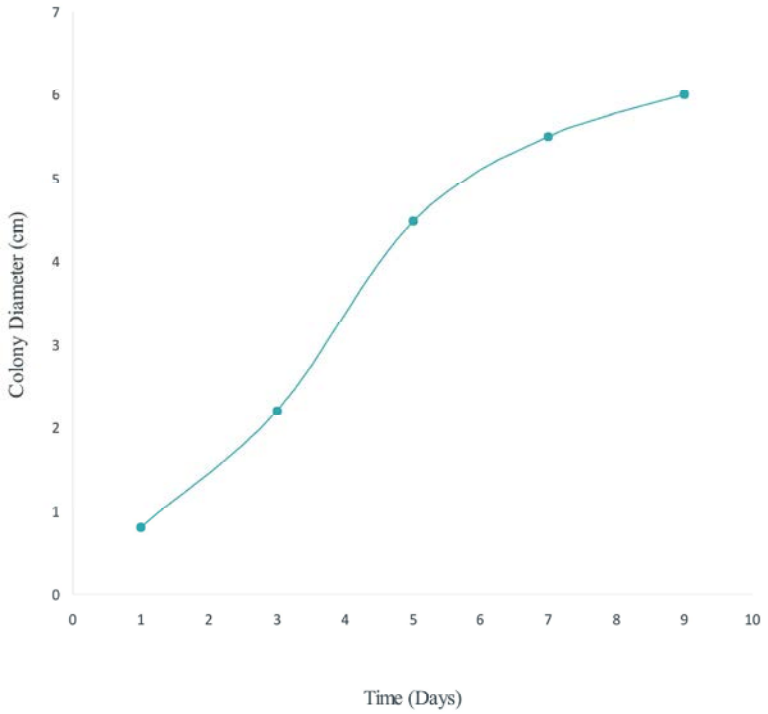

Fig. 1: Growth Curve of *Saccharomyces carlsbergensis* on Cassava Peel Extract Agar (CPEA).

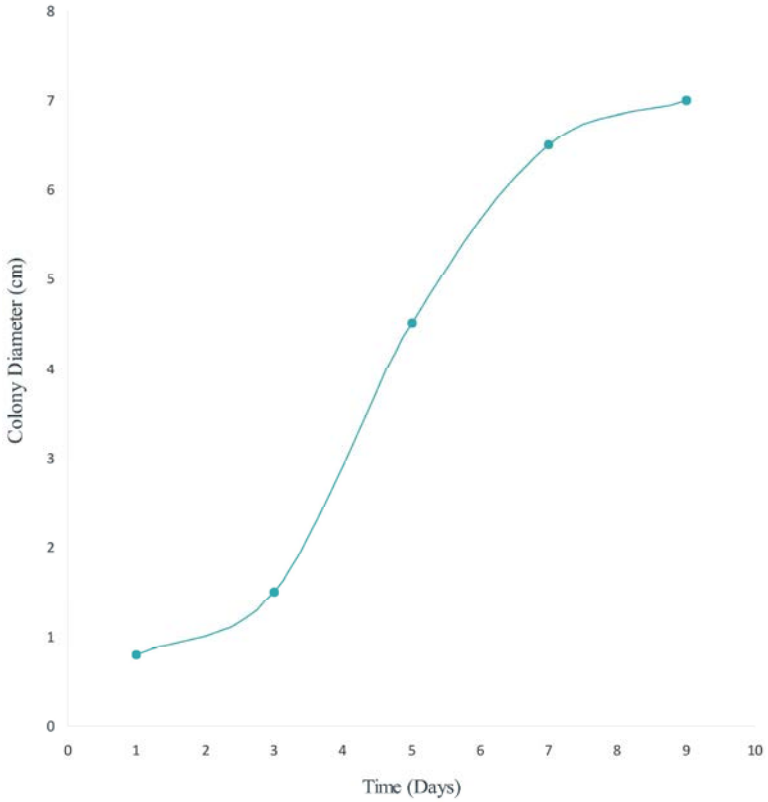

Fig. 2: Growth Curve of *Saccharomyces carlsbergensis* on Rice husk extract Agar (RHEA).

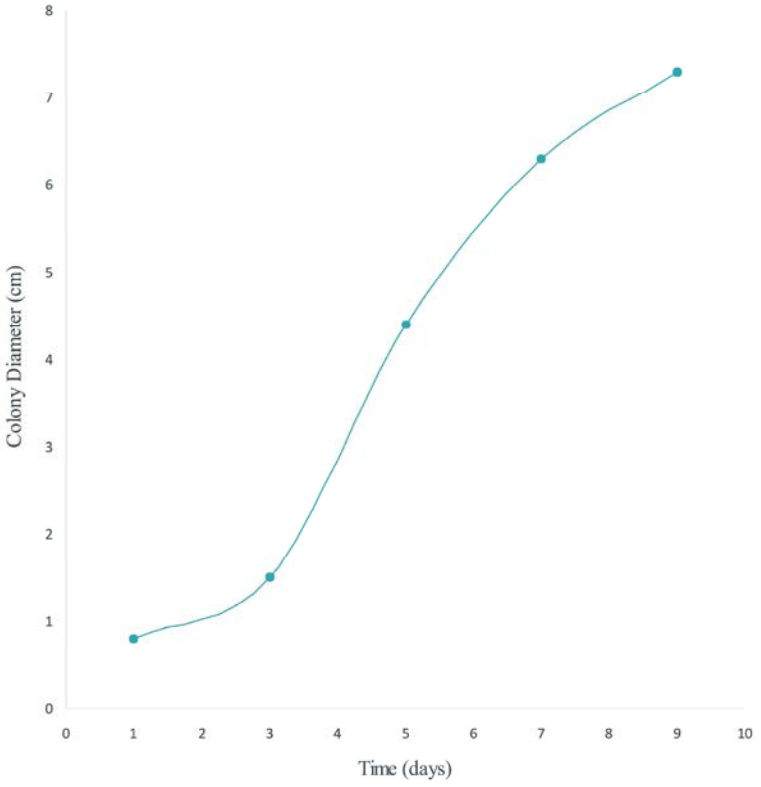

Fig. 3: Growth Curve of *Saccharomyces carlsbergensis* on Yam PeelExtract Agar (YPEA).

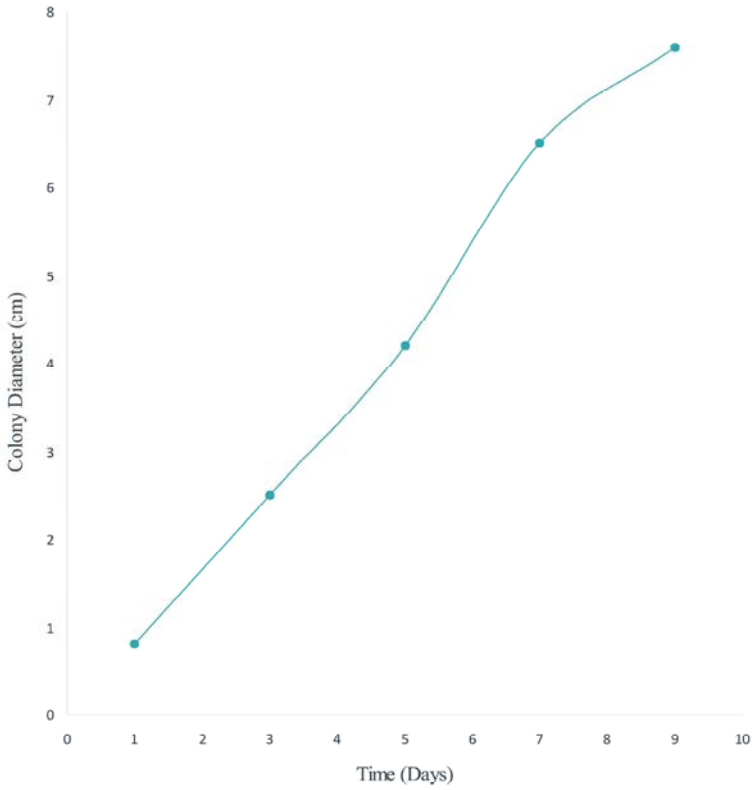

Fig. 4: Growth curve of *Saccharomyces carlsbergensis* on Potato Dextrose Agar (PDA).

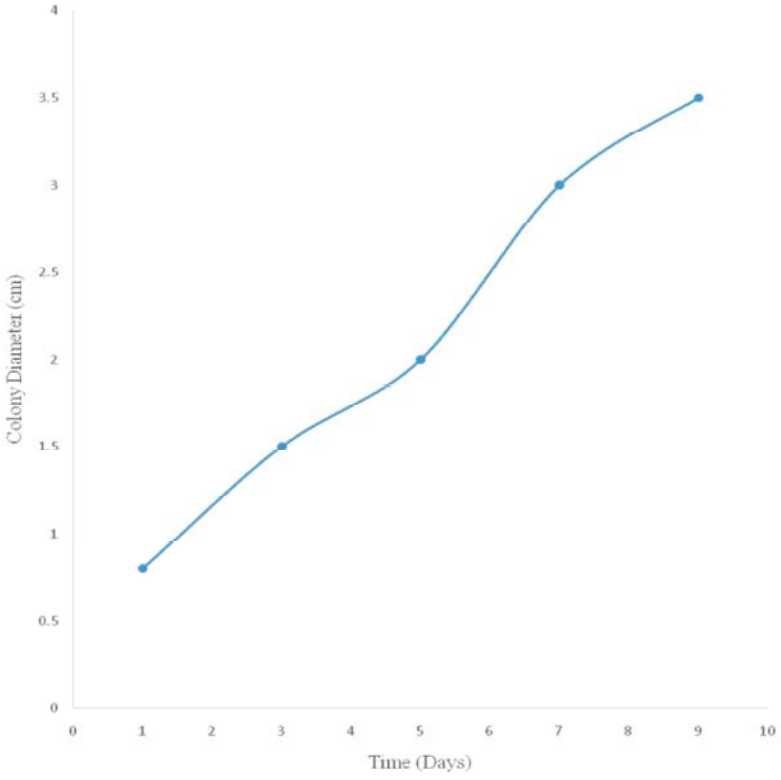

Fig. 5: Growth Curve of *Saccharomyces carlsbergensis* on Enriched Cassava Peel Extract Agar (ECPEA).

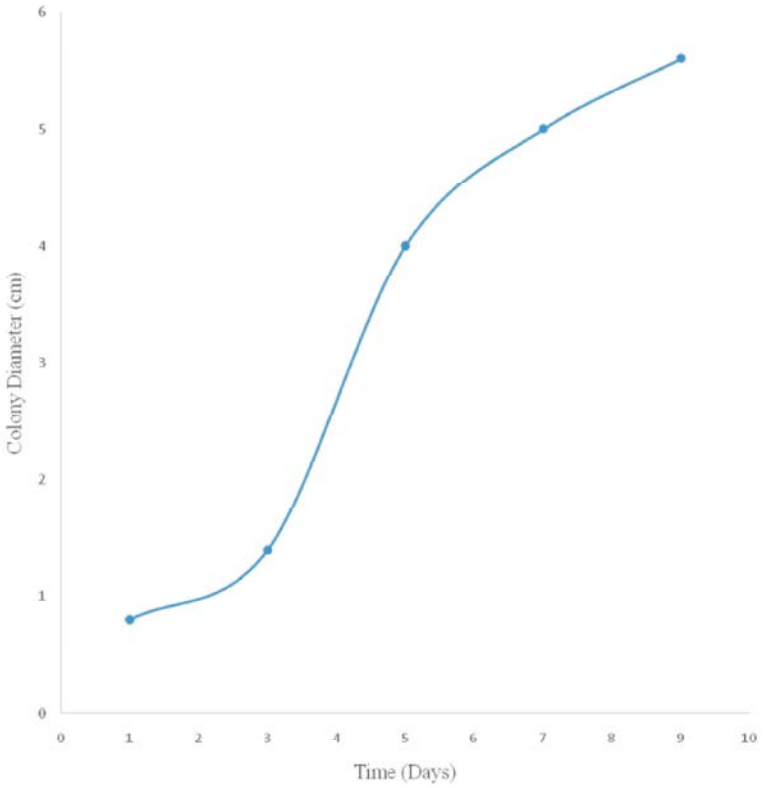

Fig. 6: Growth Curve of *Saccharomyces carlsbergensis* on Enriched Rice Husk Extract Agar (ERHEA).

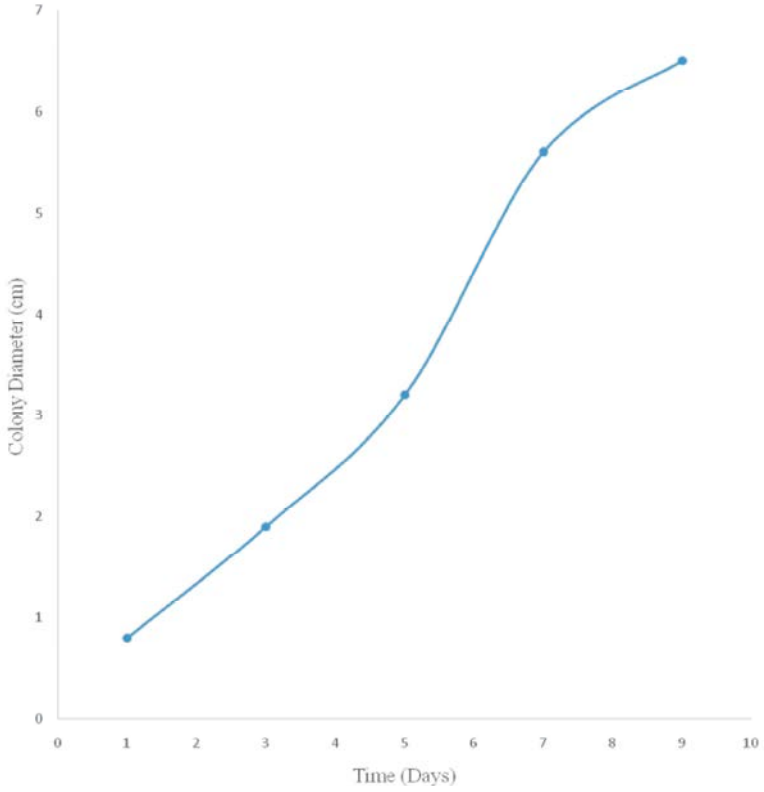

Fig. 7: Growth Curve of *Saccharomyces carlsbergensis* on Enriched Yam Peels Extracts Enriched Agar (EYPEA).

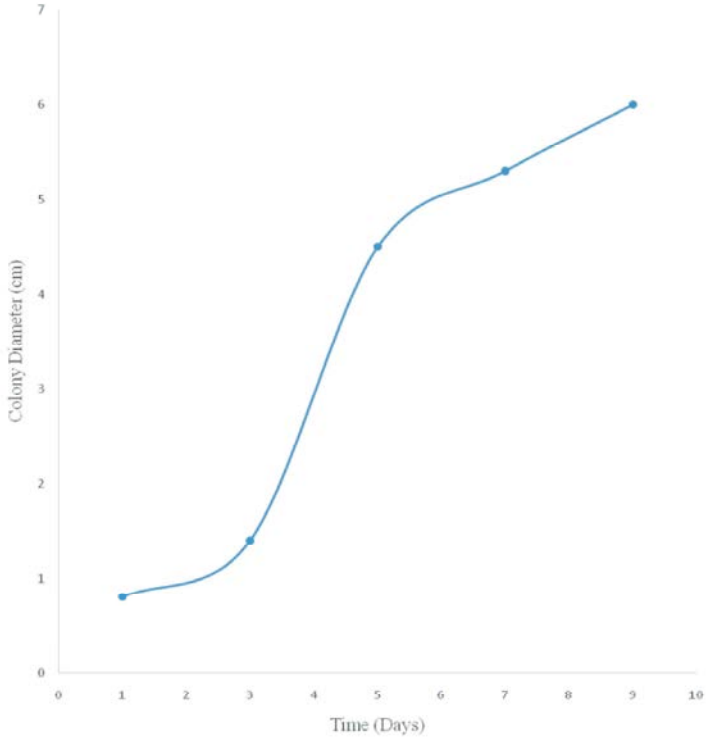

Fig. 8: Growth curve of *Penicillium* species on Enriched Rice husk Extract Agar (ERHEA).

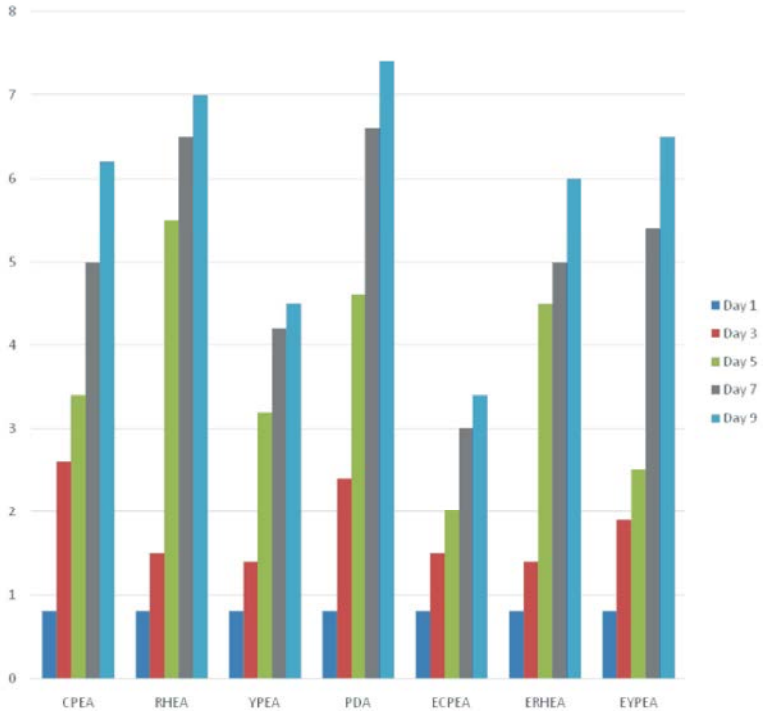

Fig. 8: Growth rate of *Penicillium species* on the formulated media and Potato Dextrose Agar
CPEA = Cassava Peel Extract Agar, RHEA = Rice Husk Extract Agar, YPEA = Yam Peel Extract Agar, ECPEA = Enriched Cassava Peel Extract Agar, ERHEA = Enriched Rice Husk Extract Agar, EYPEA = Enriched Yam Peel Extract Agar.

Results are means of triplicate determinations ± standard error. Values in the same column with the different superscripts are significantly different at ($P < 0.05$). [11].

Growth Curves of the Test Microorganisms on Different Formulated Media (A graph of Colony Diameter (cm) was plotted against Time (days).

DISCUSSION

Results of the study revealed that all the formulated media (natural formulated media and enriched media) supported the growth and sporulation of all the test microorganisms (*Saccharomyces carlsbergensis* and *Penicillium species*). This was in accordance with the findings of [8], [12], [13]. The growth of these test organisms on the formulated media implies that cassava peel, rice husk and yam peel which were used as substrates in media formulation contained the required nutrients for microbial growth (Table 1). Also, in conformity with the findings of Akiinyele & Adetuyi, [14] showed that agricultural waste materials supports the good growth of microorganisms.

The nutrients in the media included protein, carbohydrate, vitamin and minerals (macro elements and micro elements. The protein content of the formulated media must have ensured a good supply of nitrogen. Afolabi *et al*., [15] indicated that the protein content of cassava peel and yam peel was 3.72± 0.31 and 7.66± 0.31 respectively. This was quite different with the data found in this study in which protein content of yam peel extract was 0.4427^{c}± 0.0151 and that of cassava peel extract was 0.3060^{a}± 0.0020. The difference might result from the fact that different species of yam may have been used. The carbohydrate content served as additional carbon source both of which are essential for good fungal growth and was significantly different and highest in Cassava Peel Extract (0.0213^{b}± 0.0061), then lowest in Yam Peel Extract (0.0127^{a}± 0.0012), which was different from the study by Akpabio and colleagues [16].

Vitamins mainly act as coenzyme or prothetic group in cell metabolism process [9]. Vitamin C content was highest in Yam Peel Extract (7.3223^{c}± 0.0254) and lowest vitamin content of (4.0773^{a}± 0.0197) was in Rice Husks Extract. The extracts are significantly different. The mineral content of the wastes in the formulated media was probably useful for some aspects of the fungi's metabolism. The macro elements, calcium and magnesium were an inorganic cellular cation and a cofactor for certain enzyme [9]. The calcium content was highest in RHE with no significant difference in YPE and CPE. This was in conformity with the findings of Afolabi, *et al*., [15]. Manganese content was highest in CPE and least in YPE. In CPE, sodium was least and there was no significant difference in RHE and YPE (Table 1). The magnesium and zinc content was lowest in CPE and there was no significant difference between the manganese content of RHE and YPE and zinc content of YPE and RHE respectively. Iron content of the extracts RHE, YPE and CPE was significantly different in which RHE had the highest content while CPE had the lowest content.

The specific growth rate (μ) which is the increase in cell mass per unit time of which its duration is dependent upon the size of inoculum, capacity of medium and culturing conditions to support microbial growth. The results from this study showed that the ecological success of *Saccharomyces carlsbergensis* was higher on enriched fish extract media, ECPEA, ERHEA and EYPEA respectively while CPEA, RHEA and YPEA had a slower specific growth rate. The faster specific growth rate in enriched media resulted from nutrient and mineral compositions of the extract which must have aid in cell metabolism.

The mean generation time/ doubling time is the time required for microbial cells to divide. The results obtained from this study showed that *Saccharomyces carlsbergensis* proliferated more on YPEA, RHEA and CPEA than on EYPEA, ERHEA and ECPEA. Also, mean generation time of *Penicillium species* was faster on RHEA and YPEA and slower in ERHEA and EYPEA respectively. ECPEA tends to support microbial growth faster than CPEA.

CONCLUSION

Based on the findings of this study, it is concluded that cassava peel extract, rice husk extract and yam peel extract contain nutrient and minerals that can meet the nutritional requirements of fungi, thus they can be utilized as alternative substrates in the formulation of culture media for the in vitro cultivation of fungi. When compared with the conventional media it showed a good growth of the test organisms. An important advantage of these agricultural waste used in formulating the various media is that it is readily available.

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