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Isolation and cultivation of fungi with agrowastes formulated media

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ABSTRACT

This experiment was performed to test the suitability of agro waste in media formulation for fungi cultivation. Yam glucose agar (YGA), plantain glucose agar (PGA) and pineapple broth (PB) were prepared and used in comparison to a well-known commercial fungi growth medium (PDA). The formulated media supported the growth of fungi species by exposure to the environment and seeding technique. Eleven fungi species were isolated with the formulated media and are Neurospora crassa, Asteromyces cruciatus, Thysanophora longispora, Cylindrocarpon spp, Aspergillus niger, Trichoderma viride, Penicillium italicum, Aspergillus parasiticus, Gonatobotrys sp, Chrysosporium sp, Cephalosporium sp. Seeding of Aspergillus niger, Aspergillus fumigatus, Aspergillus flavus and Trichoderma viride on the formulated media also grew within three days. Antifungal activity of two plants extracts and nystatin using the formulated media was also carried out in comparison with PDA. Susceptibility of test fungi on the formulated media was comparable to potato dextrose agar. Formulated medium with pineapple on addition of agar agar did not attain solidification and therefore was converted to broth culture and as well supported the growth of fungi species.

Keywords: Agro-waste, fungi, formulation, cultivation.

INTRODUCTION

Large amount of wastes are generated every year from the industrial processing of agricultural raw materials and individual homes. Most of these wastes are used as animal feed or burned as alternative for elimination. However, such wastes usually have a composition rich in sugars, minerals and proteins, and therefore, making them useful for other processes directly or indirectly. The presence of carbon sources, nutrients and moisture in these wastes provides conditions suitable for the development of microorganisms and this open up great possibilities for their reuse. The economical aspect is based on the fact that such wastes may be used as low-cost raw materials for the production of other value-added compounds, with the expectancy of reducing production costs. The environmental concern is because most of the agro-industrial wastes contain phenolic compounds and/or other compounds of toxic potential; which may cause deterioration of the environment when the waste is discharged to the nature [1; 2; 3; 4]. The need to develop alternative media to various culture media has become imperative as the conventional media used are either not readily available or relatively expensive in most developing countries like Nigeria [5] and other developing countries of the world.

Plantain peels are utilized as animal feedstuff especially ruminants for its palatability [6]. Plantain peel contains protein contents of 80 - 110 g/kg, total dietary fibre of 400-500 g/kg and lipid content which varies from 22-109 g/kg [7]. Plantain peel is used for the production of ethanol by monoculture fermentation which removes glucose that is inhibitory to cellulose activity [8]. Carbohydrate based agricultural products such as yam is an important staple food in the diet of people in most developing countries of the tropics. Because of the high carbohydrate content of yam it can be used as raw materials to formulate media for the cultivation of micro-organisms particularly fungi, which can break down the starch to soluble sugars to serve as source of carbon and energy. In tropical countries other starch containing tubers, sweet potato (*Ipomoea batatas*), cocoyam (*Xanthosomas sagittifolium*) and

white yam (*Dioscorea rotundata*), can possibly replace the potato in a general purpose culture medium [5]. Pineapple cannery waste materials have been used as substrate for the microbial production of vanillic acid and vanillin by the use of some fungi specie [9].

The objectives of this research is basically to compose culture media from agro wastes, cultivate fungi species with the composed media and compare formulated media with conventional media (PDA) in fungal growth and antifungal susceptibility test quality.

MATERIALS AND METHODS

2.1. Collection, drying and preparation of agro wastes

The samples collected were yam peel, pineapple peel and plantain peel. The pineapple peels were collected from fruit vendors; yam and plantain peels were collected from the cafeteria 1 at Afe Babalola University, Ado Ekiti, Nigeria. The samples were kept in a sack and transported to the laboratory where they were sliced into small pieces, rinsed severally in clean water and spread on clean sacks to air dry. The dried samples were blended into smooth powder, packed in thick nylon and stored in a cool dry place before use.

2.2. Composition of cultural medium from agro wastes

Fifty grams of each powdery sample were weighed and mixed with 200 ml of 100 ^{o}C boiled sterile water, stirred with a glass rod and allowed to cool. Each mixture was filtered using muslin cloth and a filter paper. To a one hundred milliliter filtrate, 20 g of glucose and 1.5 g of agar No. 1`was added. The mixtures were sterilized at 121 ^{o}C for 15 minutes in autoclave

2.3. Cultivation of moulds

Some of the prepared agar plates of yam glucose agar (YGA) and plantain glucose agar (PGA) were exposed to different environment for 1 hour, while some were seeded with known fungi species and incubated alongside with commercial potato dextrose agar (PDA) as a positive control for 72 h.

2.4. Proximate analysis of agro wastes

The proximate compositions of the samples were determined using the standard methods of analysis of Association of Official Analytical Chemists [10]. Moisture content of the samples was determined by air oven (Gallenkamp) method at 105 ^{o}C. The crude protein of the sample was determined using micro-Kjeldahl method. Crude lipid was determined by Soxhlet extraction method using petroleum ether as extracting solvent. The ash content was determined using a muffle furnace set at 550 ^{o}C for 4 h until constant weight of ash is obtained. Crude fibre was determined using the method of [11]. The carbohydrate content was obtained by difference.

2.5. Plant extracts preparations

The plant used was *Nauclea latifolia*, the bark was scraped from the tree at Erifun village, close to Afe Babalola University Ado- Ekiti. It was thoroughly washed and rinsed in clean water after which it was shed dried in the laboratory for 2 weeks. It was ground into smooth powder with a mechanical homogenizer. 1 kg was weighed and soaked in 500 ml of ethanol for 24 h and filtered through tripled layered muslin cloth, through a Whatman's No 1 filter paper and finally through a Millipore filter. The filtrate was evaporated with rotary evaporator (RE-52A Union Laboratories England) at 45 ^{o}C to obtain semi solid extract. The extract was kept in a brown sterile bottle and stored at room temperature before use.

The fruits of *Gmelina arborea* were collected from the environment of Afe Babalola University, Ado-Ekiti. The fruits were washed, peeled to remove seeds and ground using an electric blender. The blended fruits mesocap were filtered through Whatman's No 1 filter paper and final filtration through a Millipore filter for sterility. The filtrate was stored in a closed air tight sterile brown container for further use.

2.6. Antifungal activity using composed media

The formulated yam glucose agar and plantain glucose agar were separately seeded with fungi spores and allowed to stand for one hour to be established in the medium. With a sterile cork borer, well of 4 mm was created on the fungi seeded plates. 0.5 ml of the extracts was dispensed into the respective wells. The plates were carefully carried to avoid extract splash into an inoculating hood for incubation at 28 $\pm$2 ^{o}C for 72 h. Resultant fungal growth was counted and recorded. The fungi isolated were purified by transferring to freshly prepared media which were again incubated at 27 $\pm$ 2 ^{o}C for 72 h. The pure fungi isolated were identified by the methods of [12].

2.7. Statistical Analysis

The results were expressed as mean ± standard deviation (SD) and were subjected to one way analysis of variance (ANOVA). The least significant difference (LSD) was performed for the pair wise mean comparisons, to determine

the significant treatment dose at 95% level of confidence. Values were considered statistically significant at (P<0.05).

RESULTS

Proximate analysis of the yam peel (*Dioscera alata*) had the highest percentage of starch (78.32%) among the employed agro waste products followed by pineapple with 61.02% and plantain starch percentage of 60.87%. Plantain peel (*Musa paradisiaca*) had the highest percentage of protein, crude fat, crude fibre and ash of 11.47, 1.83, 8.47 and 7.83 respectively followed by yam peel (*Dioscorea rotundata*) with 6.24, 1.56, 1.8 and 2.33 protein content respectively and the least percentage of 1.49, 0.85, 0.51 and 1.65 respectively from pineapple peel (Table 1).

Eleven fungi species were isolated with the formulated media. The identified fungi species were *Neurospora crassa, Asteromyces cruciatus, Thysanophora longispora, Cylindrocarpon spp, Aspergillus niger, Trichoderma viride, Penicillium italicum, Aspergillus parasiticus, Gonatobotrys sp, Chrysosporium sp* and *Cephalosporium sp* (Table 2, Plate 1). However, from plates exposed to different environment within Afe Babalola University campus, *Neurospora crassa, Asteromyces sp, Gonatobotrys sp, Chrysosporium sp and Cepalosporium sp* were identified (Plate 1), while the exposed formulated media in the biological science laboratory grew *Thysanophora longispora, Penicillium italicum, Cylindrocarpon spp, Aspergillus niger, Aspergillus parasiticus* and *Rhizopus stolonifer* (Table 2, Plate 1). The formulated YGA and PGA were seeded with *T. viride, A. flavus, A. fumigatus* and maximum yield growth of the above mentioned organism was observed (Plate 2).

To test for fungal susceptibility pattern using the formulated media in comparison with a known fungi cultivation medium (Potato dextrose agar), six fungi species were used. The agents used against the test fungi were two plant extract (*Gmelina arborea* and *Nauclea latifolia*) and a known antifungal (Nystatin). *P. italicum* was inhibited on formulated media (PGA and YGA) with 13 mm and 14 mm respectively by *G. arborea* extract and with 10 mm on PDA. *G. arborea* extract was not effective on *A. flavus* in both formulated media but was inhibited on PDA with 26 mm. *T. viride* was inhibited on both PGA and YGA with inhibitory zone of 16 and 13 mm respectively; and 17 mm on PDA by *G. arborea* fruit extract. On the formulated media, *A. fumigatus* was inhibited by *G. arborea* fruit extract with inhibitory zone of 11 on PGA, 16 mm on YGA and 23 mm on PDA. *R. stolonifer* was inhibited by *G. arborea* fruit extract with inhibitory zone of 12 mm on PGA, 11 mm on YGA and 8 mm on PDA. *A. niger* was inhibited by *G. arborea* fruit extract with inhibitory zone of 13 mm on PGA, 14 mm on YGA and 14 mm on PDA (Table 3). *A. flavus* was inhibited by *N. latifolia* with inhibitory zone of 8 mm on PGA and was resistant to the extract on YGA and PDA. *N. latifolia* did not create any zone of inhibition on *A. niger* and *A. fumigatus* on both PGA, YGA and PDA. *P. italicum* was inhibited on PGA and YGA with 7 mm and 8 mm zones respectively and 8 mm on PDA. *T. viride* was resist ant to *N. latifolia* extract on PDA and was inhibited with 9 mm and 11 mm on PGA and YGA respectively. *R. stolonifer* was inhibited by *N. latifolia* extract on PGA, YGA and PDA with inhibitory zone of 14, 13and 9 mm respectively. Nystatcin inhibited all the test fungi species in both formulated media from agro waste and commercial known medium (PDA). Inhibition by nystatcin on the test isolates are higher than the inhibitions created by the plant extracts. Highest inhibition with nystatcin was 32 mm against *P. italicum* on YGA and 30 mm on PDA followed by 28 mm on PGA. However, there was no significant difference with nystatcin inhibition against the test fungi isolates on both formulated and commercial fungi cultivation medium (PDA) (Table 3).

Pineapple glucose agar was converted to broth because it did not gel upon the addition of agar agar. Pineapple broth culture was observed to support the growth of *A. fumigatus, A. flavus, T. viride, P. italicum, R. stolonifer, A. niger, A. parasiticus, N. crassa, A. cruciatus* and *Gonatobotrys sp.* (Plate 3).

Table 1. Proximate composition of agro waste

Source	Starch (%)	Protein (%)	Crudefat (%)	Crude fibre (%)	Ash (%)
Yam (*D. rotundata*)	78.32±1.14a	6.24±0.26a	1.54±0.25a	1.8±0.1b	2.33±0.15b
Plantain (*M. paradisiaca*)	60.87±0.47b	11.47±0.15b	1.83±0.12a	8.47±0.12a	7.83±0.58a
Pineapple (*A. comosus*)	61.02±0.13b	1.49±0.0c	0.85±0.11b	0.51±0.07c	1.65±0.16b

Table 2. Morphological characteristics of isolated fungi

CUTURAL CHARACTERISTICS	CONIDIA ARRANGMENT UNDER THE MICROSCOPE	IDENTITY OF ISOLATES
Dark brown mycelium	Conidiospores single or in clusters bearing one to several penicillin on a single stipe proliferating sympodially after producing a cluster of phialides.	*Thysanophora longispora*
Brown	Hyphae hyaline to brown sporogenous cells	*Asteromyces cruciatus*
Dirty green colonies	Hyphae slender branched and septate. Conidiophores erect, branched near the apex to form brush-like conidia-bearing sterigmata (phallides). Conidia coloured green, i-celled, globose, to ovoid in dry basipetal chains.	*Penicillium italicum*
White mycelia with fluffy growth	Conidiophores erect, slender, hyaline, branched irregularly, terminated in slender phiallides.	*Cylindrocarpon sp*
Black conidiophores	Hyphae septate and branched, no columella, conidiophore long upright, aseptate, terminal in bulbous vesicles. Conidia globose, i-celled, black; oval in shape and spiny.	*Aspergillus niger*
Yellow conidiophores	Hyphae septate and branched, no columella, conidiophore long upright, aseptate, terminal in bulbous vesicles. Conidia globose, i-celled, black; oval in shape and spiny.	*Aspergillus parasiticus*
Mycelium white when young but turned yellowish at maturity	Branched septate conidiphores produce golden-yellow conidia in masses. Conidia i-celled short, cylindric to rounded formed acropetally.	*Neurospora crasse*
Dark green conidiophores	Hyphae erect and branching irregularly. I-celled, globose to pyriformterminal	*Gonatobotrys sp*
Green conidia	Conidiphores erect tall septate simple with terminal and interealary. I-celled hyaline ovoid to sub globose.	*Chrysosporium sp*
Grey conidia	Conidiophore and phialides slender, mostly simple conidia, hyaline, i-celled collecting in a slime drop.	*Cephalosporium sp*
White dense cotton-like mycelium that developed black spores.	Hyphae branched and coenocytic. Rhizoids formed along length of stolons. Sporangiophores formed directly above rhizoids terminate in sporangia. Well-developed umbrella-like collumellae. Spores globose to oval	*Rhizopus stolonifer*

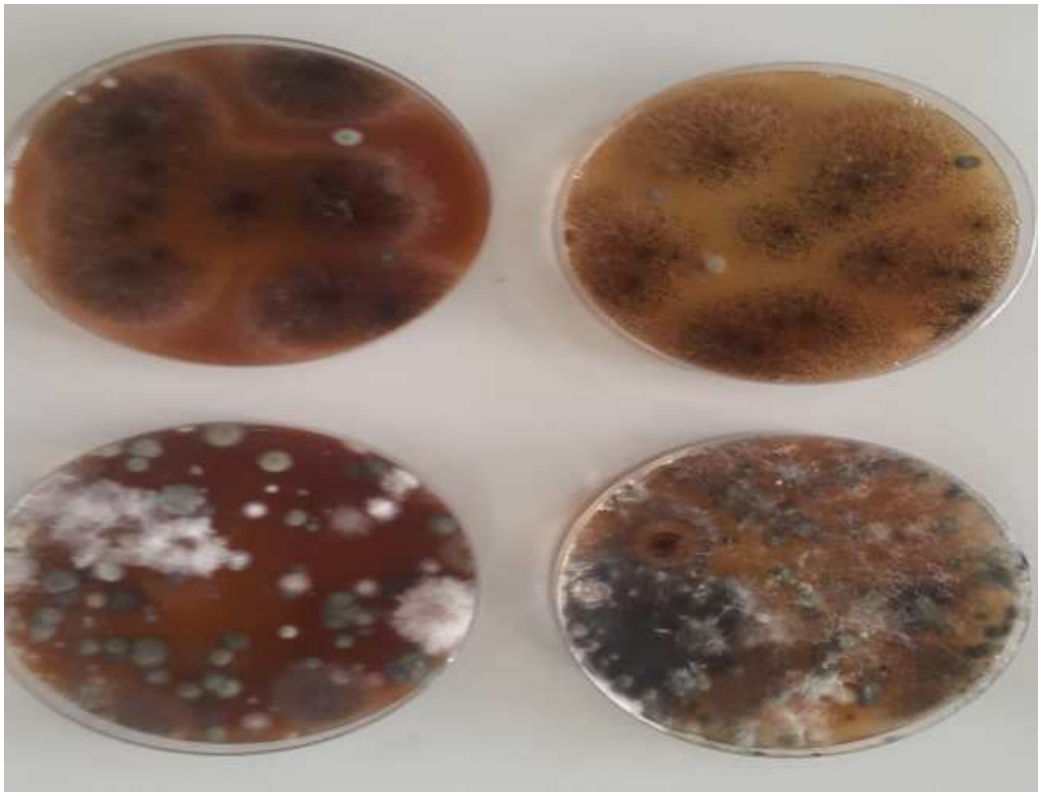

Plate 1. Photograph of environmentally exposed YGA and PGA growing different species of mould

Table 3. Susceptibility patterns of test fungi species on formulated and commercial media

Name of organism	*Gmelina arborea* (mm)			*Nauclea latifolia* (mm)			Nystatcin (mm)		
	PGA	YGA	PDA	PGA	YGA	PDA	PGA	YGA	PDA
P. italicum	13	14	10	7	8	8	28	32	30
A. flavus	-	-	26	8	-	-	21	21	20
T. viride	16	13	17	9	11	-	28	25	24
A. fumigatus	11	16	23	-	-	-	25	25	26
R. stolonifer	12	11	8	14	13	9	25	20	25
A. niger	13	14	14	-	-	-	20	18	20

Legend: Plantain glucose agar (PGA), Yam glucose agar (YGA), Potato dextrose agar (PDA).

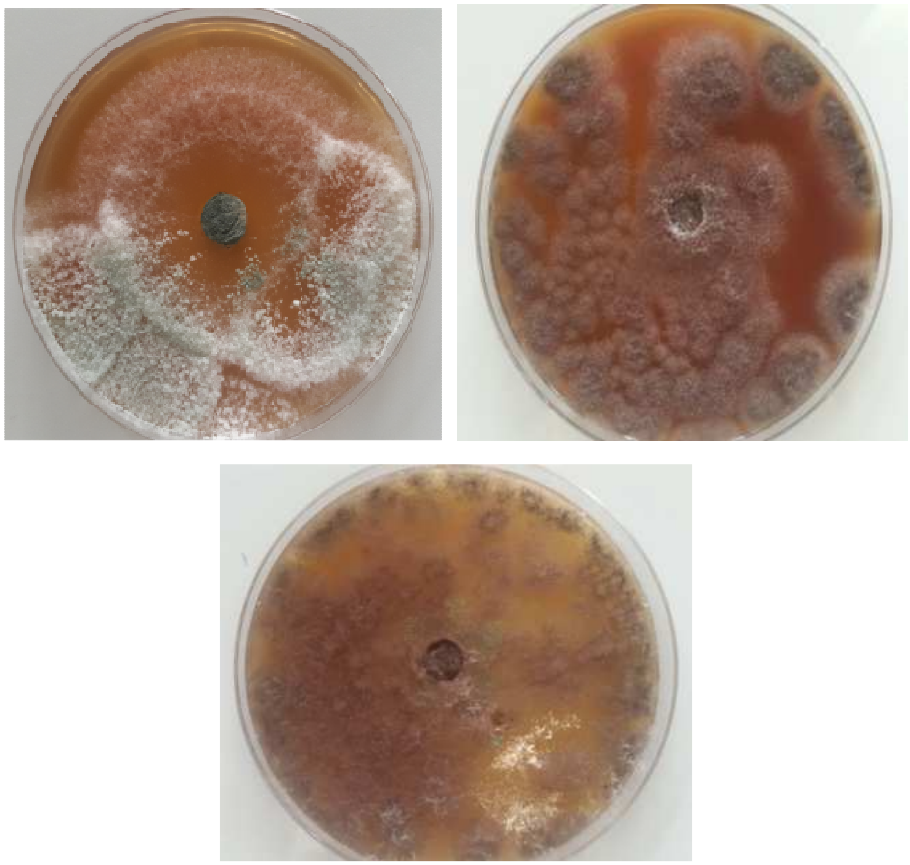

Plate 2. Photograph of seeded fungi species of *T. viride* and *A. fumigatus* on YGA and PGA on formulated media

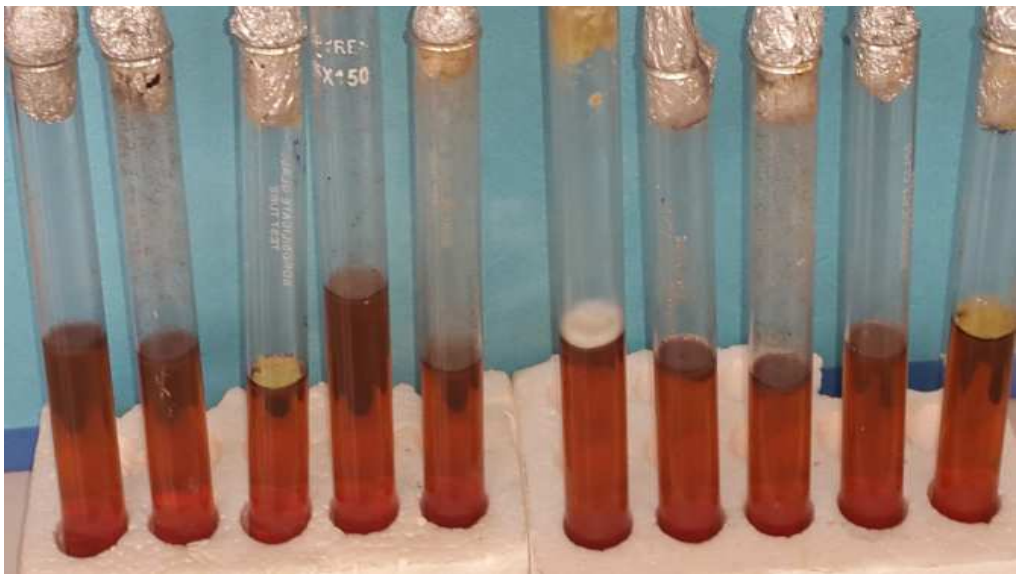

Plate 3. Photograph of Pineapple broth with fungal growth

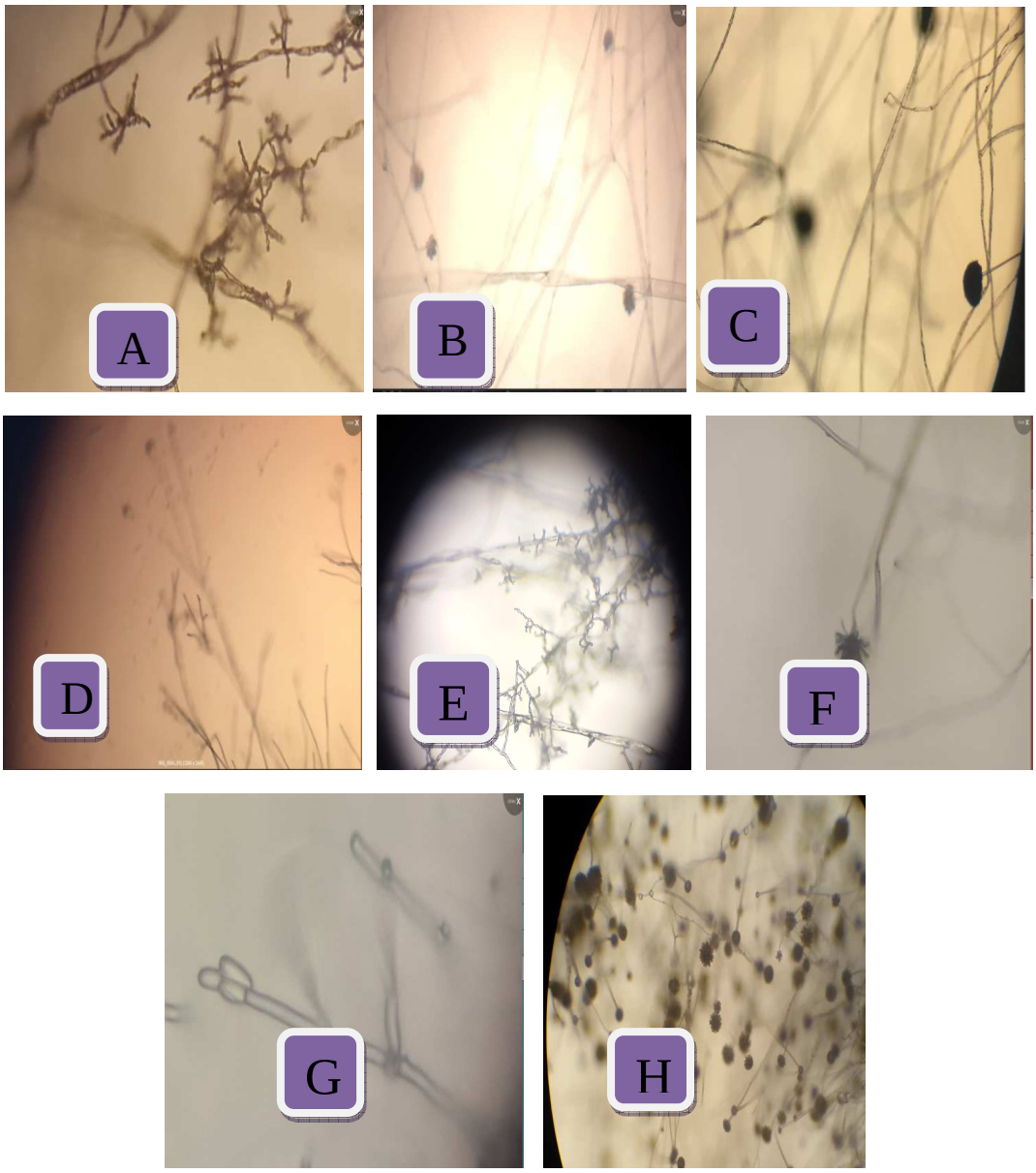

Plate 4. Photograph of A= *Chrysosporium sp.* B= *Gonaboyrys sp,* C= *Rhizopus stolonifer.* D= *Cephalosporiumsp* E=*Trichodermaviride,* F= *Asteromycescruciatus,* G= *Cylindrocarpon sp*), H=*Aspergillus fumigatus* (Mag x400

DISCUSSION

Results of the study showed that all the formulated media supported the growth of fungi. This is in conformity with the findings of [13; 14; 15; 16] 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. 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. The protein content of the formulated media must have contained high percentage of nitrogen while the carbohydrate content served as additional carbon source both of which are essential for good fungal growth.

The result of the proximate composition of yam peel, plantain peel and pineapple peel showed the following; Starch (78.32%, 60.87%, 61.02%), Protein (6.24%, 11.47%, 1.49%), Crude fat (1.54%, 1.83%, 0.85%), Crude fibre (1.8%, 8.47%, 0.51%) and Ash (2.33%, 7.83%, 1.65%) respectively which led to variation in the concentration of media components and also played a role in the overall outcome of the formulated media in good support of fungal growth. Cassava dextrose agar, corn meal dextrose agar and Palmyrah tuber medium have been reported to be better media compared to PDA for the cultivation of some fungi [13; 14; 15]. Microbiological studies depend on the ability to

grow and maintain microorganisms under laboratory conditions by providing suitable culture media that offer favourable conditions. Appreciable minerals, amino acids and carbohydrates which are the essential requirements in a suitable fungi cultivation medium were present in the agro wastes materials used for the alternative fungi growth medium. According to [17], these compounds are essential to support the growth of microorganisms without supplement. Media containing 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. The results obtained in this study with plantain glucose agar (PGA) and yam glucose agar (YGA) showed optimal spore growth which may be as a result of the nutritional constituents of the agro wastes.

The ideal medium for reference testing is mandated to be totally defined, reproducible, free of antagonists or boosters of antimicrobial action, well buffered to maintain pH, and available in both liquid and solid formulations. These criteria were met by the YGA and PGA. However, pineapple glucose agar could not gel on addition of agar No 1 to use for susceptibility test in solid form.YGA and PGA was exceptionally naturally buffered based on the results obtained without further micronutrients enhancement. The uncertainties associated with agar such as influencing the results of susceptibility testing and the substances that can stimulate growth of certain microorganisms which include biotin, thiamine, and other unknowns [18], susceptibility testing with YGA and PGA were of interest as reference medium for susceptibility testing.

Pineapple glucose agar was converted to pineapple glucose broth culture because upon the addition of agar agar, the medium could not solidify. The gelling ability of agar agar can be affected by the acidity and alkalinity of any ingredients mixed with it. The high acidic content contained in the peels could have been responsible for the neutralization of the agar agar thereby preventing it from its known gelling activity.

CONCLUSION

This study revealed that agro waste contains minerals and nutrients that can meet the nutritional requirements of fungi, thus they can be utilized as an alternative material in the formulation of culture media for the in vitro cultivation of fungi. An important advantage of the food crop peels used in formulating the various media is there readily availability, cheap if at all they are to be purchased and need no further enhancement with supplements to give maximum fungi growth. Using agro wastes for fungal cultivation media will help in reducing the amount of waste in the environment to avoid the menace wastes constitutes in some areas. With the interesting results obtained it is highly recommended that researchers and students could make use of these agro wastes in media formulation for fungi isolation.

REFERENCES

[1] JL. Zhou; RJ. Kiff.. *Journal of Chemical Technology***, 1991**, 52, 317–330.
[2] C. Haung; CP. Haung. *Water Research*, **1996**, 9, 1985–1990.
[3]SE.Bailey; TJ. Olin; RM. Bricka; DD. Adrian. *Water Research*, **1991**, 33, 2469–2479.
[4] BR. Sudha; E. Abraham. Studies on chromium (VI) adsorption using immobilized fungal biomass. *Biores. Technol*. **2003,** 87, 17–26.
[5] OC. Amadi; AN. Moneke.. *African Journal of Microbiology Research*. **2012.,** 6(21), 4527-4532.
[6] CFI. Onwuka; PO. Adetiloye; CA. Afolami. *Small Rumin. Res*, **1997,** 24, 233-237
[7] TH. Emaga; RH. Andrianaivo; B. Wathelet; JT. Tchango; M. Paquot. *Food Chemistry*, **2007,** 103, 590–600.
[8] J. Itelima; I. Onyimba; A. Ogbonna; M. Nyam; I. Nwaukwu. *Global Journal of Microbiology Research* **2014,** 2(1), 112-116.
[9] KL. Ong; BW. Tan; SL. Liew. *International Food Research Journal*, **2014,** 21(3), 953-958.
[10] AOAC., Official Methods of Analysis of the Association of Official Analytical Chemistry, 16th Edn., AOAC International, Washington, USA., **1995,** 1141
[11] F. Saura-Calixto; J. Canellas; L. Soler. *J. Sci. Food Agric*, **1983,** 34, 1419-1422.
[12] JA. Barnett; RW. Payne; D. Yarrow. Yeasts: Characteristics and Identification, Cambridge University Press, UK, **2000,** 1139
[13] G. Weststeijn; N. Okafor. *Neth. Journal.of PIant Pathology*, **1971,** 77, 134-139.
[14] AO. Adesemoye; CO. Adedire. *World Journal of microbiological Biotechnology*, **2005,** 21, 329-336.
[15] TS. Tharmila; EC. Jeyaseelan; AC. Thavaranjit. *Archive of Applied Science Research*, **2011,** 3(3), 389-393.
[16] RAO. Gabriel-Ajobiewe; BJ. Akinyele; EB. Mirrila. *J. Microbiol. Biotech. Food Sci*, **2012,** 1(4), 1136-1151
[17] B. Andualem; A. Gessesse. *Asian Pacific Journal of Tropical Biomedicine*, **2013,** 3(10), 790-797.
[18] WJ. Robbins. *J. Bot*, **1939,** 26, 772-779