Evaluation of the suitability of some common grains for the formulation and production of mycological media

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Abstract- An investigation was carried out on the growth of some fungi on laboratory formulated media using locally available grains that could be cheap and accessible. The growths of the fungi on Acha (*Digitaria exilis)*, Maize (*Zea mays)*, Rice *(Oryza sativa),* Guinea corn *(Sorghum species)* and Millet *(Pennisetum glaucum)* formulated media were compared with those on malt extract agar (MEA) and Potato dextrose agar (PDA). The test organisms including *Aspergillus niger, Penicillium natoatum, Mucor hiemelis, Candida albicans, Saccharomyces cerevisiae* showed good growth on all the formulated media. *Mucor hiemelis* grew best on maize extract agar (MzEA), *A. niger* on millet extract agar (MiEA). *P. notatum* on guinea corn extract agar (GEA) while *M. hiemelis* was best on rice extract agar (REA). Acha extract agar supported maximal growth of *P. notatum* and *M. hiemelis*. Statistical analysis of the data indicated slight variations in growth rates of the test microorganisms on all the media formulated. Proximate composition of grains was predominantly carbohydrate. However, crude protein, moisture, crude fiber and ash were present in smaller and varying amounts. Higher microbial growth observed on GEA suggests that the medium could serve as alternative growth media for fungi in place of the conventional MEA and PDA with reduced cost.

Index Terms- formulated media, grains, fungal growth,

mycological media

I. INTRODUCTION

A medium is a solid or liquid preparation containing nutrients for the cultivation of microorganisms, animal cells or plant tissue. A culture is a collection of microbiological cell growing on or in a medium [1]. A culture medium is of fundamental importance for most microbiological tests, to obtain a pure culture, grow and count microbial cells, cultivate and select microorganisms. The microbiological culture medium is a substance that encourages the growth, support and survival of microorganisms [2]. Culture medium contains nutrients, growth promoting factors, energy sources, buffer, salt, metals and gelling agents (for solid media) [1].

Many media have been designed and widely used for cultivation of various species of microorganisms. Some of them were designed based on specific growth requirements of the target species of microorganisms, while others were designed for the production of specific metabolites or for other specific processes and purposes. Some have been made selective and some other made differential [3]. Microorganisms like any other living organisms use food as a source of nutrient and energy [4].

For maximum cell growth, microorganisms require an excess supply of nitrogen and adequate aeration to achieve high cell growth and biomass yield. However, for product formation, a low concentration of nitrogen and oxygen could favor some productions [3]. Even though a lot of microbiological media have been formulated in recent times, the costs of these media and non-availability have necessitated a search for a more and probably cheaper media for the growth of these organisms.

The present work evaluated the suitability of some common grains for the formulation and production of mycological media

II. MATERIALS AND METHODS

Milled grains of Acha (*Digitaria exilis*), Guinea corn *(Sorghum bicolor*), Maize *(Zea mays),* Rice (*Oryza sativa)* and Millet *(Pennisetum glaucum)* were purchased from a local grocery market in Lafia, Nasarawa State, Nigeria. Five grams each was separately decocted in 2 l of distilled water by cooking for 30 min. Upon cooling, it was carefully filtered through a clean sheet of muslin cloth. Two hundred milliliters of the resulting extract of each grain were poured into 500 ml conical flasks solidified by adding 1.5% agar and sterilized by autoclaving at 121 ^{o}C for 15 min under pressure at 15 psi. The procedure adopted was that used by Ochei and Kolhatkar [5], in the preparation of corn meal extract agar. The pH of the medium was adjusted to 7.2 by the addition of sodium hydroxide.

Test Organisms

The test organisms included *Aspergillus niger, Penicillium notatum, Mucor hiemelis, Candida albicans, Saccharomyces cerevisiae, Staphylococcus aureus, Escherichia coli, Salmonella typhi, Klebsiella and Proteus vulgaris*. The test organisms were obtained from Microbiology Laboratory at the National Veterinary Research Institute Vom, Plateau State, Nigeria.

Cultural Analysis

The test fungi were point inoculated using sterile wire loop on the media and in incubated for 5 days. The radial growth on each medium was measured using a transparent ruler. Bacteria were inoculated on the media and incubated according to the method adopted by Arora and Arora. [6]. The growth was monitored through colony counts.

Determination of proximate compositions of the grains

Proximate analysis was carried out and the moisture content, crude fiber, ash content, crude protein and total carbohydrate of the different grains were determined.

Statistical Analysis

Statistical analysis (using SPSS) was carried out using analysis of variance (ANOVA).

III. RESULTS

Growth of *Aspergillus niger* on the media

The diameter of radial growth of the organism increased progressively (Fig. 1). In Day 1, there was an equal growth of *A. niger* on Maize extract agar (MZEA) and Potato dextrose agar (PDA). The growth of the organism was highest in these two media. Malt extract agar (MEA) and Rice extract agar (REA) had equal diameter while Acha extract agar (AEA) showed the least growth. The diameter of growth increased through the Day 5 where GEA showed the highest growth followed by PDA, MEA, MIEA, REA, MZEA and AEA respectively (Fig. 1).

Growth of *Candida albicans*

In day 1 of the experiment, *C albicans* grew on MEA and PDA and their rates of growth were equal. This was followed by growth on GEA. There was no growth on MZEA, MIEA, REA and AEA. The trends in growth continued to Day 5, and GEA was the best medium for the growth of this organism (Fig. 2).

Growth of *Saccharomyces cerevisiae* on the media

In day 1, MEA showed higher growth than PDA. There was no growth on other media. In day 5 of the experimental period, GEA displayed the highest growth followed by PDA, REA, MZEA, MEA, AEA and least on MIEA (Fig. 3).

Growth of *Penicillium notatum* on the media

There was no growth on MIEA, GEA and AEA on the first day of inoculation. However, the fungus exhibited growth through day 2 to day 5. The radial diameter of growth of *P. notatum* was highest on GEA in day 5 (Fig. 4).

Growth of *Mucor hiemelis* on the media

The organism responded to the media within 24 h showing equal growths on MEA, PDA, GEA and REA. In day 5, growth was highest on MZEA while MIEA and PDA showed the least growth (Fig. 5). Figure 6 shows a comparison of the growth of fungi on the formulated media.

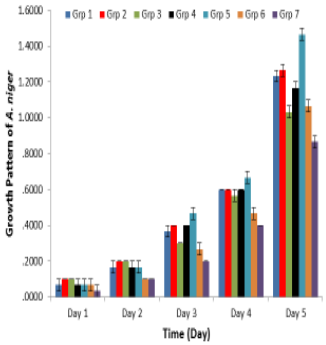

Fig. 1. Time course of *A. niger* growth on all the media.

Note: Grp1-MEA, Grp 2-PDA, Grp 3- MzEA, Grp 4-MiEA, Grp 5-GEA, Grp 6-REA, Grp 7-AEA.

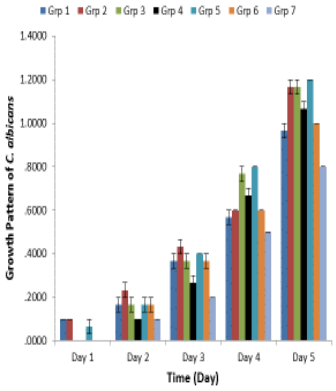

Fig. 2 Growth of *C. albicans* on all the media.

Note: Grp1-MEA, Grp 2-PDA, Grp 3- MzEA, Grp 4-MiEA, Grp 5-GEA, Grp 6-REA, Grp 7-AEA.

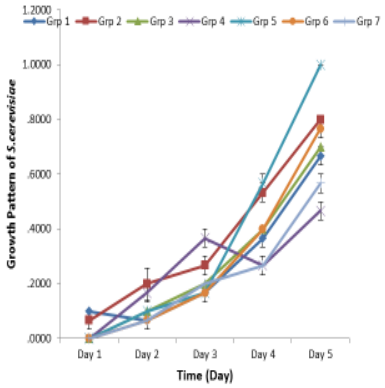

Fig. 3. Growth Performance of *S. cerevisiae* on all the media.
Note: Grp1-MEA, Grp 2-PDA, Grp 3- MzEA, Grp 4-MiEA, Grp 5-GEA, Grp 6-REA, Grp 7-AEA.

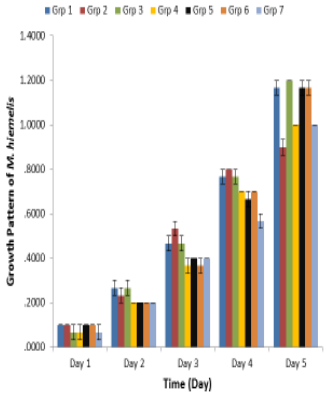

Fig. 5. Growth of *M. hiemelis* on all the media.
Note: Grp1-MEA, Grp 2-PDA, Grp 3- MzEA, Grp 4-MiEA, Grp 5-GEA, Grp 6-REA, Grp 7-AEA.

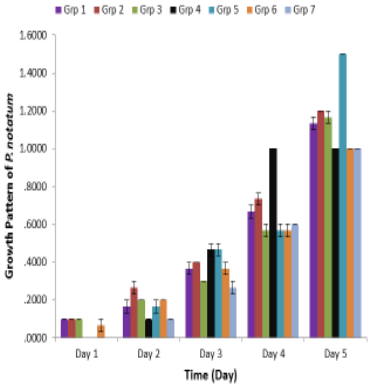

Fig. 4. Time course of growth of *P. notatum* on all the media.
Note: Grp1-MEA, Grp 2-PDA, Grp 3- MzEA, Grp 4-MiEA, Grp 5-GEA, Grp 6-REA, Grp 7-AEA.

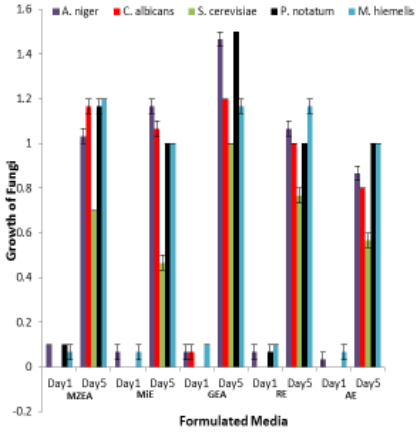

Fig. 6. Comparison of growth of fungi on the formulated media

Table 1. Proximate compositions of the grains

Grain	Moisture (%)	Crude Fiber (%)	Ash Content (%)	Crude protein (%)	Total Carbohydrate Content mg/100 ml
Acha	10.28	0.8	1 ± 0.001	8.61	46.38
Guinea corn	10.73	1.4	1 ± 0.001	7.39	73.9
Maize	10.65	3.2	2 ± 0.01	10.04	56.52
Millet	11.25	2.0	2 ± 0.01	11.77	60.87
Rice	9.92	ND	2 ± 0.01	9.06	55.0

IV. DISCUSSION

Some selected microorganisms encountered in routine microbiological work were tested for their ability to grow in our laboratory formulated media. Their growth patterns including the number of counts and the diameter of radial growth were used as indices of performance. The results of the measurements were compared to one another and with the standard media for similarities and differences.

There was a progressive increase in the growth of *A. niger* in all the media tested. The zone diameters observed on day 1 and day 5, were the smallest and largest respectively in all the media. PDA and MEA are the conventional media used in the routine cultivation of fungi. Comparing the growth of *A. niger* in the formulated and the conventional media, in day 2, the growths on the conventional media were significantly higher. However, this trend did not continue until the end of the experiment. The growths on the formulated media at certain points showed no statistically significant difference ($P \geq 0.05$) with the conventional media used as the control. This implicates the formulated media as being equally good for the cultivation of the fungus.

The fungus showed the highest growth on GEA on day 5 in comparison with its growth on MEA, MzEA, MiEA, REA and AEA. Hence, *A. niger* grows higher in a more carbohydrate medium. Proximate analysis showed that Guinea corn has 73.9 mg/100 ml of carbohydrate with nitrogen as low as 4.38 %. This could probably explain why the fungus grew better on GEA.

Candida albicans grew better on the conventional media than on our formulated media on the first 24 h of incubation. However, the growth of the organism on MEA, PDA and REA were related as the experiment progressed. In day 3, MEA, PDA, GEA and REA had similar radial growth patterns whereas growths on MzEA and MiEA were different. In day 5, a significant difference arose between MEA and PDA. There was also a slight difference between MzEA and MiEA as does between MiEA and GEA, GEA and REA as well as REA and AEA. GEA offered the most conducive environment for the growth of *C. albicans*. *C. albicans* is an oval budding yeast cell which produces pseudohyphae when the buds continue to grow. The fungus is normally present in skin and mucosa.

According to Arora and Arora. [6], blood culture provides most reliable evidence of systemic infection and that *C. albicans* grows well on Saboraud Dextrose Agar (SDA) and on ordinary bacteriological media at 25 ºC – 35 ºC which have a similar composition to the locally formulated media. Ochei and Kolhatkar [5], reports that corn meal agar is a useful mycological medium which is used for the production of chlamydospores. Corn meal agar composed of corn meal extract 1.0 g, distilled water 500 ml and 7.5 g agar. This composition is similar to the composition of our formulated media.

Mucor hiemelis growth showed no significant difference on all the media during the experimental period. In day 5, the organism showed no difference in its growth on all the media although, the highest growth was observed on MzEA. The high sugar content and the considerable high amount of moisture in the raw materials used in the preparation of media are responsible for the growth of mucor Ochei and Kolhatkar [5]. *Penicillium notatum* also showed slight differentiation. There was no significant difference in growth amongst MEA, PDA and MzEA. In day 5, differences in growth occurred between MEA and PDA, PDA and MzEA, MzEA and MiEA, MiEA and GEA, GEA and REA while REA and AEA had no significant difference in their growth phase on the media. However, the highest growth of the organism was observed on GEA. *P. notatum* occurs as saprophytes in the soil and decomposing organic debris where a large amount of carbon is produced Arora and Arora. [6]. Guinea corn has a considerable high amount of carbon as an element in its nutrients which constitutes a relatively high amount of carbohydrate.

Saccharomyces cerevisiae showed slight differentiation in its growth on the media. There is no significant difference in its growth at the beginning of the experiment. However, as the experiment progressed, slight differences were observed. *Saccharomyces cerevisiae* showed the highest growth on GEA. The average growth was low compared to growth of the other test microorganisms. This could be explained by the fact that even though the nutritional requirements of the organisms may be met, genetic factor has its effect on the organism. Consequently, according to Prescott *et al.* [7], when nutrients remain plentiful haploid and diploid cells undergo mitosis to produce haploid and diploid daughter cells respectively. The growth rate of fungi on formulated and conventional media increased progressively each day.

The present study on fungi is in agreement with Amienyo *et al.* [8], who carried out an investigation on the growth of some fungi in a laboratory formulated medium using acha (*Digitaria exilis*) and compared the growth with those on a standard fungal media. Their test fungi included *Rhizoctonia solani* and *A. niger*.

At the end of the experimental period, GEA offered the best environment for the growth of *A. niger*, *C. albicans*, *P. notatum* and *S. cerevisiae*. While MzEA offered the most favorable environment for the growth of *M. hiemelis*, MiEA demonstrated a most favorable environment for the growth of *P. vulgaris* while NA and MEA as standard media offered a most suitable environment for the growth of *S. aureus*, *S. typhi*, *E. coli* and *P. vulgaris*. However, MiEA stands out as the best media. GEA can be used as a selective medium for fungi while MiEA can be used as a selective medium for bacteria. According to Ochei and Kolhatkar [5], an amino acid which is a component of protein

was found to be a suitable growth factor for bacteria. Millet was shown to have a considerable high amount of crude protein of 11.77 g.

Carbohydrates dominated the proximate compositions of all the grains. Similarly, carbon was the predominant element. In the formulation of the media, additional water was added and this increased the moisture content and water activity (a_w) of the media. Millet has the highest moisture content as compared to others. High or low moisture contents of the grains could be attributed to the drying and storage conditions Acha, Guinea corn, maize and rice. The very low moisture content of rice and acha could be suggested that the two grains lose a considerable amount of water during storage resulting in a longer shelf life [9]. Maize has the highest crude fiber compared to millet, guinea corn and acha. This could be as a result of the size of the grain. Maize, millet and rice have equal amounts of ash while acha and guinea corn have lower values.

V. Conclusion

All the test organisms showed growth on the standard media and on the formulated media. However, the differences in the growth on the media were not significant. Because of the high cost of MEA, NA, PDA and other microbiological media and their non-availability in many parts of the country, one advantage of the locally formulated media is the availability of the grains (Acha, maize, rice, guinea corn and millet). The substrates from the extracts are available locally and cheap. Also, the method of preparation of MzEA, AEA, MiEA, REA and GEA are simple and easily adaptable.

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