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Production of Mycological Media using Local Raw Inedible Materials

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ABSTRACT:
The use of local inedible raw materials in the formulation of mycological laboratory medium which is cost effective was carried out using brewer's spent grain (BSG) samples gotten from Intafact Breweries Onitsha, Anambra state. The BSG was mixed with 5g of cow blood, 20g of *Ede mmuo* (*Caladium bicolor*), 5g of *Fiofio*, and 20g of agar agar as a gelling agent. The other media was prepared with the same materials but without agar agar. The formulated media was inoculated with *Aspergillus niger* as the test fungus. The two different media were compared for gowth diameter. The fungal organism grew faster on agar agar medium with wider colony diameter. On agar-agar medium, the length on the first day for the four plates were 1cm each. Second day for the four plates include; plate 1(15.0mm), plate 2(20.0mm), plate 3(10mm), and plate 4(15mm). Third day include; plate 1(30mm), plate 2(40mm), plate 3(32mm), and plate 4(35mm). Fourth day include; plate 1(60mm), plate 2(50mm), plate 3(35mm), and plate 4(60mm). Non agar-agar medium, the length on the first day for the four plates were 3mm each. Second day for the four plates include; plate 1(10.0mm), plate 2(11.0mm), plate 3(11.0mm), and plate 4(10.0mm). Third day include; plate 1(12.0mm), plate 2(14.0mm), plate 3(13.0mm), and plate 4(13.0mm). Fourth day include; plate 1(15.0mm), plate 2(16.0mm), plate 3(14.0mm), and plate 4(15.0mm).

Keywords: *fiofio, Caladium bicolor,* Cow blood, brewer's spent grain, *Aspergillus niger*

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INTRODUCTION

Mycological media are basal media to which antifungal agents may be added for checking their effect on fungi or bacteria to render them selective for isolation and cultivation of fungi. Mycological agar is used while working with pathogenic fungi. Earlier media for fungi generally relied on an acidic pH to make the media less suitable for the growth of many bacteria (Gregory *et al*., 2008). A wide range of media are used for growing fungi. Most mycologists develop preferences for certain types of media based on experience and peculiarities of the type of fungi that are routinely grown. Media will affect colony morphology and color, whether particular structures are formed or not, and may affect whether the fungus will even grow in culture. For example, some fungi lack the necessary enzymes to utilize different carbon sources. All fungi require several specific elements for growth and reproduction (Gregory *et al*., 2008, Agu *et al.*, 2016).

Raw materials in form of food industrial waste have been channelled towards the production of industrial, commercial and pharmaceutical products that include energy and fine biochemical products (Miller, 1998; Agu *et al.*, 2016). The waste of a company is seemingly the raw material for another.

Brewers spent grain (BSG) are major by-product of the beer industry, representing around 85% of the total by product generated. BSG contains 17% cellulose, 28% non-cellulosic polysaccharide and 38% lignin (Mussato and Robert, 2005). BSG can serve as an adjunct in human nutrition due to high protein content. They also been used in feeding both ruminant and

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monogastric animal. It has also been shown to be an excellent media for microbial growth of yeasts and moulds.

Cajanuscajan(L.) Millsp. (Leguminosae) known as "*Fiofio*" in Igbo, otiiliin Yoruba and pigeon pea in English (Aiyeloja and Bello, 2006) is native to India which is the world's largest producer. It is also grown in Africa and the Americas, and has been suggested to be one of Africa's drought-tolerant crops referred to as 'orphan crop' because it falls into the group of least researched crops world-wide (Odeny, 2007). It serves both as a food and forage crop. *C. cajan*can be combined with cereal to make a wellbalancedhuman food. It is used in combinationwith soya bean to produce one of the popularfermented, flavouring product soy sauce. *C. cajan*contains minerals like potassium, magnesium, calcium and is significantly low in sodium. The low sodium content might be one of the reasons it is employed in enthno-medicine for the treatment of hypertension (Lawal, 2012). Italso has vitamins such as vitamin A, niacin and small amount of thiamin, riboflavin, folate andpantothenic acid (Akande*et al*., 2010).

Fungi are a group of eukaryotic spore-bearing, achlorophyllous organism that generally reproduce asexually and sexually. They are important in nutrient recycling department of nature (Khalid *et al*., 2006). Fungi due to their competitive saprophytic ability expressed by fast mycelial growth, spore production, presence of efficient and extensive system of powerful enzymes are able to utilize complex polysaccharides and protein as their carbon and nitrogen sources (Laleye*et al*., 2007). Microorganisms need nutrients, a source of energy and certain environmental conditions in order to grow and reproduce. In the environment, microorganisms adapt to the habitats most suitable for their needs while in the laboratory, these requirements

must be met by a culture medium (Simin, 2011). Microorganisms can obtain energy directly from sunlight while carbon can be made available in organic form such as carbohydrate. For a microbiological media to fulfill its specific purpose, it must contain all the substances and compounds necessary for the growth and reproduction of the organism.

Potato Dextrose Agar is a common medium used to grow fungi in laboratories. This is a basic medium composed of dextrose, potato extract and agar. As the readily available culture media are expensive, there is a need to find alternative media or reduce the amount of agar added during the preparation of culture media in order to reduce the overall cost involved. Few of the studies described below have focused on addressing this issue. Arnan-Parh*et.al.*, 2010 has worked on cowpea as a cost effective alternative culture media for the growth of bacteria. There are reports that used starch sources such as sago, palmyrah tuber flour, tubers of sweet potato and cassava as alternative growth media for fungi (Tharmilla *et al*., 2011). Further there are also reports using vegetables as an alternative source for preparing culture media for the growth of fungi and bacteria (Deivanayaki and Irutharayaj, 2012). This study is therefore aimed at producing mycological media from local raw inedible materials to minimize cost production.

The aim of this study was to produce a cheap mycological growth medium using local raw inedible materials as growth factors as well as investigate the feasibility of using these raw materials as an inexpensive but effective alternative culture medium against conventional mycological media for isolating and identifying molds.

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Materials and Method

Sample Collection

Brewer's spent grains were collected from Intafact Breweries Company, Onitsha, South East Nigeria and transported to NnamdiAzikiwe University Microbiology laboratory.*Ede mmuo* (*Caladium bicolor*) and *Fiofio* were purchased from Eke-Awka market and also transported to the laboratory for further analysis.The cocoyam plant, Heart of Jesus (botanical name: *Caladium bicolor*) was independently identified by Prof. Okigbo R.N. and Prof. Izundu A.I. of Botany Department of NnamdiAzikiwe University.

Medium Preparation

Medium Formulation using Agar Agar as Gelling Agent

Exactly 5g of spent grain was mixed with 5g of cow blood, 20g of *Ede mmuo* (*Caladium bicolor*), 5g of *Fiofio*, and 20g of agar agar. They were all mixed with 200ml of distilled water. The conical flask was plugged tightly with cotton wool and then sterilized in an autoclave at 121^{O}Cfor 15minutes at 15psi and dispensed into four petri dishes.

Medium Formulation Using *Ede mmuo*(*Caladium bicolor*) Starch as Gelling Agent

Exactly 5g of spent grain was mixed with 5g of cow blood, 20g of *Ede mmuo* (*Caladium bicolor*), and 5g of *Fiofio*. They were all mixed with 180ml of distilled water. The conical flask was plugged tightly with cotton wool and then sterilized in an autoclave at 121^{O}C for 15minutes at 15psi and dispensed into four petri dishes.

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Collection of the Test Microorganisms

Pure culture of the test fungal isolate (*Aspergillus niger*) stored in agar slant were obtained from Applied Microbiology and Brewing Laboratory, Nnamdi Azikiwe University, Awka, Nigeria.

Inoculation and Incubation of the Test Fungus

The test fungus was inoculated into the eight mycological media using the pure culture obtained from the microbiology laboratory. The plates were incubated at room temperature for 48hrs. Growth on the plates were measured.

Characterization of the Test Fungi

The improved slide culture technique of Agu and Chidozie (2021) was employed in this study. A sterile glass slide was placed on the bottom of a sterile petri dish. With the aid of a sterile 2 ml syringe, 0.5 ml of the molten Saboraud Dextrose Agar (SDA) maintained at 45 ^{O}C in a water bath was dispensed on the sterile glass slide. The cover of the petri dish was replaced and the molten agar allowed to gel. Upon gelling, a sterile inoculation needle was used to inoculate the agar bump with a small amount of fungus at the centre of the bump. Therefter, a heat-sterilized coverslip was laid just over the agar bump without pressure. The plates were incubated at room temperature for 3 to 5 days depending on the growth rate of the fungus. When desired growth was observed, few drops of Lactophenol cotton blue stain was dropped at the interface of the developing cultures on the slide and the coverslip so as to preserve the integrity of the culture and allowed to permeate the entire culture before viewing under the microscope. Referencing was done using Fungal Atlases (Barnett and Hunter, 2000; Watanabe, 2002; Ellis *et al*., 2007).

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Results

Table 1: Identification of the fungal test organism

Colony morphology	Microscopy	Identity
Colonies were compact with white or yellow basal felt covered by a dense layer of dark-brown to black conidial heads.	Conidial heads are large (up to 3 mm by 15 to 20 µm in diameter), globose, dark brown, becoming radiate and tending to split into several loose columns with age. Conidiophore stipes are smooth-walled, hyaline or turning dark towards the vesicle. Conidial heads are biseriate with the phialides borne on brown, often septate metulae. Conidia are globose to subglobose (3.5-5 µm in diameter), dark brown to black and rough-walled	*Aspergillus niger*

Table 2: Comparison of Growth Diameter of *Aspergillus niger* on Medium Formulated Using Agar Agar as Gelling Agent

DAYS	PLATE 1(mm)	PLATE 2(mm)	PLATE 3(mm)	PLATE 4(mm)
1	**10.0**	**10.0**	**10.0**	**10.0**
2	**15.0**	**20.0**	**10.0**	**15.0**
	30.0	**40.0**	**32.0**	**35.0**
4	**60.0**	**50.0**	**35.0**	**60.0**

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Table 3: Comparison of Growth Diameter of *Aspergillus niger* on Medium Formulated Using *Ede Mmuo (Caladium Bicolor)* Starch As A Gelling Agent

DAYS	PLATE 1(mm)	PLATE 2(mm)	PLATE 3(mm)	PLATE 4(mm)
1	3.0	3.0	3.0	3.0
2	10.0	11.0	11.0	10.0
3	12.0	14.0	13.0	13.0
4	15.0	16.0	14.0	15.0

DISCUSSION

Microbiological media provide an artificial means for the growth of microorganisms because they contain some essential nutrient that could enhance their growth and metabolism (Tharmila*et al*., 2011). These media are used for selective and differential cultivation of microorganisms. All fungi require a relatively large amount of carbon source and most utilize carbohydrate more readily than other carbon compounds (Hawksworth, 2001).

The two different media used gave positive growth for the test organisms. The agar-agar medium growth length is more than that without agar-agar. The result of this study revealed that the media formulations are nutritious and has all it takes to support the growth of fungi which is in line with the study of Adesemoye and Adedire,(2005) where different fungal species were successfully cultured in alternative media using different cereals to replace potato in potato dextrose agar (PDA). Okorondu *et al*., 2011 also used Garri agar to isolate fungi species. The results of this study are also consistent with the work of Tharmila *et al*., 2011 where local

materials (Sago, Palmyrah tuber and Cassava tuber) were successfully used to grow fungi. The result of this study revealed that Brewer's spent grain, *Fiofio*, and *Ede mmuo* (*Caladium bicolor*) are nutritious and has all it takes to support the growth of fungi; also the pH of BSG is a little acidic. This contributed to the growth of fungi and inhibited the growth of contaminant bacteria (Okorondu *et al*; 2011) that could grow on the more conventional media. The growth rate of organism in A.A plate supercedes that of non A.A plate but the growth on non A.A still shows that there is a possibility of new media to replace synthetic media. This new media is cheaper and the raw materials can easily be sourced.

CONCLUSION

The relative performance of fungal growth on the alternative media, when compared with the conventional media illustrated a good growth. The different media gave good result but the use of agar agar as a gelling agent is not too economical and does not reduce cost of production, so it is not advisable to use agar agar.

Due to the concentrations of these raw materials, they possess nutrients that support the growth of fungal organisms. When mixed at the right proportion, these raw materials are cost effective since fungal growth media can be obtained in the environment and at a cheaper rate. Though the availability of these raw materials can be challenging, provision should be made to ensure their availability when needed.

Therefore, these alternative media can be used for the cultivation of fungi which is found to be cost effective in the face of the costly nature of getting conventional media.

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