

Research Article

Formulation of Fungal Media from Local Plant Materials

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A culture medium is a liquid or gelled substance that promotes microorganism growth in the laboratory. This study is aimed at exploring various local plant sources for fungal media formulation. The local plant materials used in this study were grains of *Zea mays*, seeds of *Glycine max*, and boiled and peeled *Dioscorea dumetorum* tubers. These plant materials were dried separately in the oven at 60°C, pulverized individually, measured in different proportions, and poured separately into conical flasks containing distilled water at various proportions to evaluate the potential of the fungal media formulation. The best formulation was derived from *Z. mays* (5 g), *D. dumetorum* (2.5 g), and *G. max* (2 g) in 50 ml of sterile distilled water (M₅Y_{2.5}S₂). It was then autoclaved at 121°C for 15 mins at 15 psi and dispensed in various Petri dishes. Media characteristics such as gelling ability and consistency were compared with Sabouraud dextrose agar (SDA) as the standard media. Isolates of *Aspergillus niger* and *Penicillium expansum* were used; these were derived from deteriorating yam and bread and cultured on the media. The colony diameter, aerial growth, and sporulation of the test fungal isolates were ascertained. When compared to SDA (8.92 ± 0.10), the colony diameter of *A. niger* on M₅Y_{2.5}S₂ (8.90 ± 0.10) did not differ significantly ($P = 0.05$). This is also applicable in the case of *P. expansum* on M₅Y_{2.5}S₂ (8.93 ± 0.06) when compared with SDA (8.92 ± 0.07). There was a significant difference ($P = 0.05$) in the sporulation of *A. niger* (M₅Y_{2.5}S₂ 1.76 ± 0.06) when compared with SDA (1.70 ± 0.06). This is also applicable in the case of *P. expansum* (M₅Y_{2.5}S₂ 2.73 ± 0.06) when compared with SDA (2.70 ± 0.0). The fungal isolates showed luxuriant growth in both media. M₅Y_{2.5}S₂ performed favorably in comparison to SDA in relation to all the parameters studied. M₅Y_{2.5}S₂ is recommended for use in fungal culture and will save funds used for the importation of media.

1. Introduction

The ability to maintain and grow microorganisms in a laboratory environment is a crucial step in the development of microbiological studies. A nutrient substrate that is used to grow microorganisms in a laboratory is referred to as a culture medium. It can be either gelled or liquid media [1, 2].

The availability of nutrients, a good carbon source, a suitable pH, a suitable temperature, relative humidity, inorganic salt, and water, the availability of enzymes, vitamins, mineral elements such as phosphorus and sulfur, and a nitrogen source are all required for microorganism growth. These factors are also important in controlling the development of harmful microorganisms, which can lead to food

spoilage and diseases. Having a good understanding of these conditions can also help in the development of beneficial microbes that can also help in studying them. To develop a medium for microbial growth, consideration should be given to the availability of energy and carbon sources [3]. Sunlight can provide energy for microorganisms, and carbon can also be made available in a variety of organic forms, such as carbon dioxide. In order to accommodate the diverse needs of their organisms, a culture medium should be designed to meet these requirements [4, 5].

Despite the fact that most fungi prefer to grow on natural media, synthetic media are frequently used in bioassay work. This suggests that the need for alternative media is not overemphasized. The use of locally formulated media

can help reduce the reliance on imported media and provide researchers with an alternative, cost-effective method of conducting their research [6].

The high cost of synthetic media used for the production of microorganisms is a major issue that affects the quality of microbiological research in developing countries. This issue is also a serious problem for schools since a lack of culture media can prevent them from carrying out effective studies. Although synthetic media are commonly used to grow microorganisms, fungi prefer to grow in media that are made from natural materials. This study is aimed at finding out about plant materials that could be used to support the growth of fungi [7].

2. Materials and Method

2.1. Collection of Samples. Seeds of *Zea mays* L. and *Glycine max* L. and tubers of *Dioscorea dumetorum* (Kunth) Pax used in this study were obtained from a retail outlet in Nkpor, Anambra State, Nigeria.

2.2. Identification and Authentication. The plant samples used in this study were authenticated and identified by a taxonomist at the Nnamdi Azikiwe University, Awka, Nigeria.

2.3. Processing of Plant Samples. These procedures below were carried out by adopting the method of Kwoseh et al. [8].

Zea mays seeds were cleaned and ground. 1 kg of ground maize was blended into a paste using a clean blender. Using a cheesecloth, the paste was strained into a clean plastic bucket, and the solution obtained was topped with three liters of sterile distilled water. The maize starch, also known as “akamu,” was left in the laboratory at room temperature for 24 h before the supernatant was poured off to obtain a clean starch paste. To increase solubility, the starch was broken into pieces and oven-dried for 3 h at 60°C before being crushed into powder. For the study, dried starch powder was used.

Glycine max seeds were washed thoroughly with clean water and dried for 3 h at 60°C in the oven. To enhance solubility, 1 kg of the dried sample was blended into a powder. The dried ground material was sieved until it reached flour consistency, which was then used for the study.

Dioscorea dumetorum tubers were peeled, washed with clean water, and boiled for 30 mins. The boiled yam was oven-dried for 3 h at 60°C. 1 kg of the dried yam was ground into powder to enhance solubility. The dried powder was sieved until it reached flour consistency, which was then used for the study.

2.4. Test Organism. The test fungal isolates were *Aspergillus niger* var. and *Penicillium expansum* Link.

2.5. Media Preparation

2.5.1. Evaluation of the Gelling Properties of Media Formulated with Plant Samples. These plant samples (*Zea mays*, *Dioscorea dumetorum*, and *Glycine max*) at different concentrations in three replicates were placed in a conical flask containing

distilled water at various volumes and autoclaved at 121°C and 15 psi for 15 mins.

The media's ability to gel was then determined by dispensing them into sterile Petri dishes under aseptic conditions at a temperature of 27°C. 20 ml of the medium was dispensed into each Petri dish. After 45 minutes, the gelling ability was determined by rotating and examining the Petri dish containing the medium. Petri dishes containing Sabouraud dextrose agar (SDA) served as controls.

2.5.2. Culturing of Test Organisms on Selected Media. The fungal isolates were collected from the deteriorating piece of yam and bread and cultured on the fungal medium using a sterilized wire loop.

2.6. Evaluation of Media. These procedures below were carried out by adopting the methods of Kwoseh et al. [8] and Adesemoye and Adedire [7].

2.6.1. Evaluation of the Gelling Properties and Consistency of Formulated Culture Media and SDA. The gelling properties of the mycological culture media were observed after the Petri dishes containing the medium were turned upside down and observed after 45 mins. Additionally, a visual evaluation of the various media's consistency on plates was done.

2.6.2. Comparing the Physical Characteristics of SDA and Formulated Media. A pH/mV/TEMP meter (Suntex SP-701 model) was used to measure the pH levels of the media both before and after autoclaving, and the final pH value was recorded. The colour, presence of particles, and clarity of the media were observed.

2.7. Evaluation of the Diameter of the Test Fungi Colony. At seven days of growth, the colony diameter was measured in cm by drawing two diagonal lines perpendicular to one another across the base of the 9 cm-diameter Petri plate, meeting where the fungal plugs were positioned. The radial growth of the fungi on each plate was measured along the diagonals with a ruler, and the mean per plate was calculated.

2.8. Estimation of Test-Fungi Sporulation. On the seventh day of fungal growth, a 5 mm-radius cork borer was used to cut three discs from each plate. Each disc was taken from the plate about 1 cm from the center. Then, using sterile distilled water, three samples from each culture plate were bulked into a 20 ml McCartney bottle. The dilution was performed five times, with a final serial dilution of 10⁻⁵. The spores on a haemocytometer slide were counted under a microscope, and the mean spore counts (x) per unit disc were square root transformed and used for analysis.

2.9. Assessment of Test Fungi for Aerial Mycelia Growth. The aerial growth was measured based on the appearance of the vegetative growth of the test fungi. The outcome was determined by a panel of three people based on visual observation.

TABLE 1: Evaluation of *Zea mays*, *Glycine max*, and *Dioscorea dumetorum* for gelling properties.

Samples	Plate	Concentrations (g)	Water	Acronym
<i>Zea mays</i>	1	<i>Zea mays</i> (2.5) + (2.5) <i>D.dumetorum</i> + (0.5) <i>Glycine max</i>	30 ml	M _{2.5} Y _{2.5} S _{0.5}
+	2	<i>Zea mays</i> (3.0) + (2.5) <i>D.dumetorum</i> + (0.5) <i>Glycine max</i>	30 ml	M ₃ Y _{2.5} S _{0.5}
<i>Dioscorea dumetorum</i>	3	<i>Zea mays</i> (3.5) + (2.5) <i>D.dumetorum</i> + (1.0) <i>Glycine max</i>	40 ml	M _{3.5} Y _{2.5} S ₁
+	4	<i>Zea mays</i> (4.0) + (2.5) <i>D.dumetorum</i> + (1.5) <i>Glycine max</i>	40 ml	M ₄ Y _{2.5} S _{1.5}
<i>Glycine max</i>	5	<i>Zea mays</i> (5.0) + (2.5) <i>D.dumetorum</i> + (2.0) <i>Glycine max</i>	50 ml	M ₅ Y _{2.5} S ₂
SDA (control)	6	SDA (2.0)	30 ml	

Three replicates of each plate were used for the study. Key: *Dioscorea dumetorum* = Y; *Zea mays* = M; *Glycine max* = S.

3. Results and Discussion

Microbiological studies depend on the ability to culture and nurture microbes under favorable conditions in the laboratory [1]. The average molecular weight directly determines the viscosity of an agar broth at a constant temperature and concentration [9]. In the context of this reference, the increased viscosity of the medium was the cause of the difficulty in dispensing media with a higher starch concentration. In this study, the formulated media that were sticky and lost their viscosity after a short period of time were discarded and not used for further research.

Some of the media formulations were found to be less viscous and easier to dispense into Petri dishes, but they later failed to solidify and liquefy. This may be a result of the amount of carbohydrate or starch in the plant components used in the formulation of the media. Also, some of the media were molten; they simply smeared the flask lining, making it difficult to dispense them. The media solidified but formed lumps, and they also lost their consistency with a bad odour afterwards, so they were discarded (Table 1). Kwoseh et al. [8] reported that the difficulty in dispensing the formulated media and lump formation after autoclaving the media is probably due to the high concentration of carbohydrates.

M₅Y_{2.5}S₂, like most other formulated media, showed strong gelling ability but lost its gel after a few days (Table 1). According to Lupano and González [10], this is most likely caused by the pH dropping, which frequently happens after autoclaving the media. After autoclaving, the pH of all the media decreased, supporting Lupano and González's [10] conclusion that a pH decrease reduces gel stability.

From Table 1, M₅Y_{2.5}S₂ was selected from the above formulated media studies and used in comparing the standard medium SDA based on its strong gelling ability, stability, and consistency. M₅Y_{2.5}S₂ media also remained stable within eight days, becoming neither watery nor emitting any bad odour, supporting Adubofour's [11] conclusion that a medium needs to be stable enough to support common procedures like plating and streaking.

The physical properties of M₅Y_{2.5}S₂ and SDA showed average pH values of 5.15 and 5.05, respectively, which is conducive to fungal growth and sporulation (Table 2). This confirms Ritchie's [6] findings that fungi prefer a slightly acidic reaction pH of 6-6.5, while bacteria prefer a neutral pH of around 7.

TABLE 2: Comparing the physical characteristics of M₅Y_{2.5}S₂ and SDA (control).

Media	pH value	Colour	Particles	Clarity
M ₅ Y _{2.5} S ₂	5.15	Cream	NIL	Opaque
SDA	5.05	Yellow	NIL	Transparent

TABLE 3: Mean diameter (cm) values of *Penicillium expansum* and *Aspergillus niger* on SDA and M₅Y_{2.5}S₂.

Media	Fungal isolates	
	<i>Aspergillus niger</i>	<i>Penicillium expansum</i>
SDA	8.92 ± 0.10	8.90 ± 0.10
M ₅ Y _{2.5} S ₂	8.92 ± 0.07	8.93 ± 0.06

From Table 3, the colony diameters of *A. niger* and *P. expansum* on both M₅Y_{2.5}S₂ and SDA media were not significantly different ($P > 0.05$), and this supports the observation by Adubofour [11]. The radial growth of the test isolates was very rapid, which may have been influenced by nutritional factors, as the starches from the yam and maize could have provided the fungus with an additional carbon source, as suggested by Onwueme [12].

On the M₅Y_{2.5}S₂ medium, remarkable mycelia growth and sporulation were observed under experimental conditions; however, to varying degrees, this is probably due to additional supplements like phytic acid, dietary minerals, and vitamin B in *Glycine max* seeds (Table 4). This supports the findings of Adesemoye and Adedire [7] and Tharmila et al. [13] that variation in the concentration of media components also influences the overall outcome of the formulated media.

There was sporulation of *A. niger* and *P. expansum* on both media, although there was a statistically significant difference in the mean number of spores produced on both media ($P < 0.05$). Both media generally supported very good aerial growth of the isolates, although M₅Y_{2.5}S₂ media exhibited profuse growth.

From Table 5, it was observed that *A. niger* and *P. expansum* had very good aerial growth on both M₅Y_{2.5}S₂ and SDA media, although M₅Y_{2.5}S₂ media exhibited abundant growth. Starch provides 35% carbohydrate and 1% mineral matter, according to Onwueme [12]. As a result, the starch may have provided the medium with additional carbon sources that encouraged healthy growth.

TABLE 4: Mean sporulation values of *Penicillium expansum* and *Aspergillus niger* on SDA and M₅Y_{2.5}S₂.

Media	Microorganisms	
	<i>Aspergillus niger</i>	<i>Penicillium expansum</i>
SDA	1.70 ± 0.06	2.70 ± 0.0
M ₅ Y _{2.5} S ₂	1.76 ± 0.06	2.73 ± 0.06

TABLE 5: Aerial mycelia growth of *Penicillium expansum* and *Aspergillus niger* on SDA and M₅Y_{2.5}S₂.

Media	Microorganisms	
	<i>Aspergillus niger</i>	<i>Penicillium expansum</i>
SDA	++++	++++
M ₅ Y _{2.5} S ₂	++++	++++

Key: ++: good growth; +++: very good growth; ++++: profuse growth.

4. Conclusion

The majority of locally developed media, particularly M₅Y_{2.5}S₂, demonstrated some degree of gelling ability. *Zea mays* failed to gel even after a long period and therefore was blended with *Discorea dumetorum* flour; *Glycine max* flour was also added to increase the nutrient supplement available to the fungi.

Formulated culture media with higher concentrations of starch were sticky and thus not easily dispensed into Petri dishes; they liquefied after a few days, emitting a bad odour. *P. expansum* and *A. niger* were able to grow and reproduce on the M₅Y_{2.5}S₂ and SDA media used in the study. For mycological culture media preparation, the locally developed media (M₅Y_{2.5}S₂) used in the study can be used to reduce the cost and overdependence on synthetic media.

Data Availability

All required data are attached to the document.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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