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Assessment of The Potential of Sugarcane Bagasse as Substrate in the Growth of Selected Fungal Species

Muhammad, M.A.*[1], Machido, D.A.[1] and Inabo, H.I.[1]

[1]Department of Microbiology, Faculty of Life Sciences, Ahmadu Bello University, Zaria, Nigeria.

*Corresponding Author: murjanahmad@gmail.com; +2348065292667

Abstract

The cost of culture media is continuously rising at a very fast rate, thus, there is need to devise means by which low-cost media of comparative performance could be produced. This study was aimed at assessing the potential of sugarcane bagasse (one of the major waste biomasses in Northern Nigeria) to directly support fungal growth, as it contains a considerable amount of nutrients for growth. Isolates of Aspergillus flavus and Trichophyton species obtained from the Department of Microbiology, Ahmadu Bello University, Zaria were used for this study. The sugarcane bagasse was dried, cut into smaller pieces then ground into finer particles (1-2mm diameter) using a milling machine and sterilized in Petridishes by autoclaving. The fungal isolates Aspergillus flavus and Trichophyton species were resuscitated and authenticated using standard procedures, and then inoculated aseptically onto duplicate plates of the sugarcane bagasse and other minerals, and incubated at a temperature of 25°C for 7days. It was observed that the sugarcane bagasse supported a luxuriant and rapid growth of both fungi without any visible form of contamination. It was also observed that A. flavus grew more luxuriantly, consuming about 52.5% of the original amount of the bagasse than Trichophyton species, which consumed 32.5%. Sugarcane bagasse as a growth medium does not essentially meet the needs for growth of every microbe, most especially bacteria, and therefore, it can be used effectively to minimize contamination by microorganisms other than fungi. It can therefore be used as a good alternative and cheaper medium for the detection of fungi in the laboratory. This could also be an easier and cost-effective means by which wastes such as sugarcane bagasse could be removed from the environment, as opposed to using hazardous methods like burning, which could cause air pollution.

Key Words: Aspergillus flavus, Trichophyton species, Culture Media, Sugarcane bagasse, Waste Product

INTRODUCTION

Most or all microbiological processes carried out in laboratories which involve microorganisms of all classes involve the use of microbiological media for the growth of such microorganisms. However, most of these media used especially by research laboratories and students of Microbiology are obtained commercially from companies that produce them at high prices which are often not affordable to students (Sidana and Farooq, 2014), especially from economically backward nations. Hence, most students and researchers find a lot of difficulties during their research due to challenges related to the purchase and subsequent production of culture media. There is therefore need to design new microbiological media which are both efficient and affordable (Sidana and Farooq, 2014). This may be achieved by using materials that are very much available especially in the form of low cost agro and horticultural wastes (Sharma *et al.*, 2006; Ahluwalia and Goyal, 2007) and by-products such as sugarcane bagasse (Kong *et al.*, 2014), rice husk, sawdust, coconut husk, oil palm shell, neem bark, etc. (Sharma *et al.*, 2013) as raw materials for microbial media production. Although not all potentially applicable waste materials have been systematically examined yet, sugarcane bagasse could also be adapted as an alternative material for designing a new medium (Dhokpande and Kaware, 2013) as it is readily available in large volumes in the environment. Use of sugarcane bagasse as a source of nutrients for growth of the microbial biomass would also help to reduce the nuisance caused by this material to the environment, as it constitutes a major source of environmental pollution as mentioned by Sharma *et al.* (2013) and Sidana and Farooq (2014).

Sugarcane Bagasse is a by-product of sugarcane (*Saccharum officinarum*). It is the fibrous residue left or generated after the sugar has been extracted from the cane (Kadam, 2000; Parani *et al.*, 2016).

It may be formed either by chewing directly, or due to industrial processing to extract sugar (Zafar, 2015). As an agricultural by-product, it is generated in large quantity in countries that produce it, and requires no special treatment or processing before it is used as substrate material (Parani *et al.*, 2016). Sugarcane bagasse mainly composes of cellulose, hemicellulose, pentosan, and lignin (Sharma *et al.*, 2013), as well as other components like ash, moisture and reducing sugars (Zafar, 2015).

Sugarcane bagasse is very useful, and has been employed in many agricultural and industrial processes. Some of these include; the production of fuel grade bioethanol for various industrial activities (De Souza *et al.*, 2012; Zafar, 2015), as local manure and component of animal feed (Kadam, 2000; Irfan *et al.*, 2011; Zafar, 2015; Parani *et al.*, 2016); also as raw material for generating electricity (Kadam 2000; Irfan *et al.* 2011), pulp and paper production (Daud *et al.*, 2007) generating fermentation products (Ojumu *et al.*, 2003; Bhattacharya *et al.*, 2011) as well as precursor in the activation of carbon for industrial purposes (Lori *et al.*, 2007). In recent years, several studies have been carried out to explore the use of sugarcane bagasse as a low-cost adsorbent in bioremediation processes such as been carried out to explore the use of sugarcane bagasse as a low-cost adsorbent in bioremediation processes such as in the removal of heavy metals and hydrocarbons from industrial effluents (Ahluwalia and Goyal, 2007; Sharma *et al.*, 2013; Kong *et al.*, 2014; Parani *et al.*, 2016). Another important use of sugarcane bagasse is its use as a source of nutrient for microbial growth (Ojumu *et al.*, 2003; Cunha *et al.*, 2012; Oliveira *et al.*, 2012; Parani *et al.*, 2016). Sidana and Farooq (2014) carried out a study where the capacity of sugarcane bagasse was tested as a potential medium for designing fungal cultures.

This study was carried out, aiming to assess the possibility of using sugarcane bagasse in supporting the growth of *Aspergillus flavus* and *Trichophyton* spp., specifically to determine the proximate composition of the sugarcane bagasse, ability of the sugarcane bagasse in supporting growth of *Aspergillus flavus* and *Trichophyton* species, and also devise possible measures of reducing environmental pollution by using some of the waste materials as useful raw materials.

MATERIALS AND METHODS

Collection of Samples

Sugarcane bagasse:

Sugarcane bagasse was collected from Samaru, Zaria in new polythene bags and taken to the Laboratory of the Department of Microbiology, Ahmadu Bello University Zaria for analysis. The sample was air-dried and chopped into smaller pieces using a pair of scissors, and there after ground using a milling machine to finer particles.

Fungal isolates

Fungal isolates used in this study were obtained from the Department of Microbiology, Ahmadu Bello University, Zaria, which were originally isolated from refinery effluent in previous studies by Machido *et al.* (2014). The fungal isolates were then confirmed and re-authenticated by following standard procedures (Prescott *et al.*, 2002). These include sub-culturing on freshly prepared Potato Dextrose Agar (PDA) plates, which allows for easy and quick growth of the organisms, followed by microscopic examination. The authenticated isolates were sub-cultured onto fresh Sabaroud Dextrose Agar (SDA) slants for future use.

Determination of the Proximate Components of Sugarcane Bagasse Sample:

The proximate composition of the sugarcane bagasse was determined using standard methods as outlined by the Association of Official Analytical Chemists, AOAC, (2010); where by its major components such as moisture, protein, lipid, ash content, carbohydrate, cellulose, hemicellulose, and lignin were determined.

Assessment of Growth of *Aspergillus flavus* and *Trichophyton* species on Sugarcane Bagasse:

This was done by weighing 20g of sugarcane bagasse in each of the three (3) 20cm diameter Petridishes containing *Aspergillus flavus*, *Trichophyton* species as well as the negative control (followed by addition of 20mL of 200mg/L of each of ammonium nitrate (NH_4NO_3), magnesium sulphate ($MgSO_4$) and potassium hydrogen phosphate (K_2PO_4) solution, thoroughly mixed with the sugarcane bagasse to serve as supplement nutrients. The Petridishes and their contents were autoclaved at 121°C for 15mins under a pressure of 151b/sq. inch, after which suspensions containing 3.0×10^4 spores per mil each of *Aspergillus flavus* and *Trichophyton* species were inoculated onto duplicate plates of the freshly sterilised sugarcane bagasse, while another plate prepared the same way was left un-inoculated to serve as control. All inoculated plates were incubated for 7days under aerobic conditions in a dark cupboard under ambient laboratory conditions. The cultures were monitored continuously for a period of 7days, after which they were harvested, dried and weighed using the top loading balance to obtain the dry weight of the partially utilised bagasse.

RESULTS:

Proximate Composition of Sugarcane Bagasse:

The proximate analysis of sugarcane bagasse shows that it consists of the following components: Carbohydrates, which had the highest percentage (64.7%), followed by crude fibre (26.02%), while fat content was the lowest (0.2%) (Table 1).

Table 1: Proximate Composition of the Sugarcane Bagasse

Components	Amount (%)
Carbohydrates	64.7
Crude fibre	26.02
Fat	0.2
Protein	4.81
Ash	0.75
Moisture	3.52

In figure 1, the crude fibre components of the sugarcane bagasse analysed showed cellulose as having the largest content (46.5%), while ash content was lowest with 0.75%.

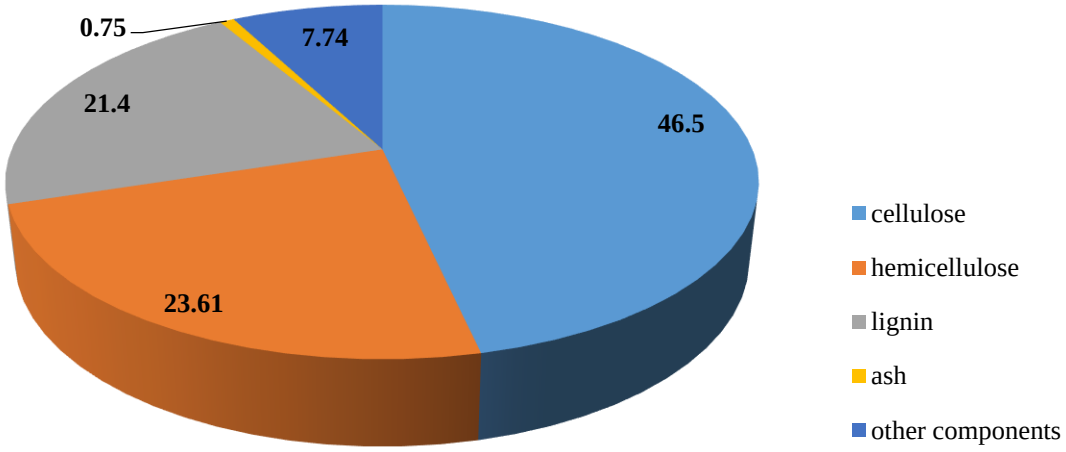

Fig 1: Crude Fibre Components of Sugarcane Bagasse

Sugarcane Bagasse Capacity to Support the Growth of Experimental Fungi:

Plates containing sugarcane bagasse, which were inoculated with *Aspergillus flavus and Trichophyton* species showed a luxuriant growth of the organism, indicating that sugarcane bagasse was able to support growth of the isolates (Plates II and III) when compared to the un-inoculated bagasse (Plate I). Growth of *Aspergillus flavus* on the sugarcane bagasse was observed to be faster and more luxuriant after the period of incubation than that of *Trichophyton* species (Table 2), as it was able to utilise 52.5% of the substrate, higher than what was utilised by *Trichophyton* sp. (32.5%) (Table 2)

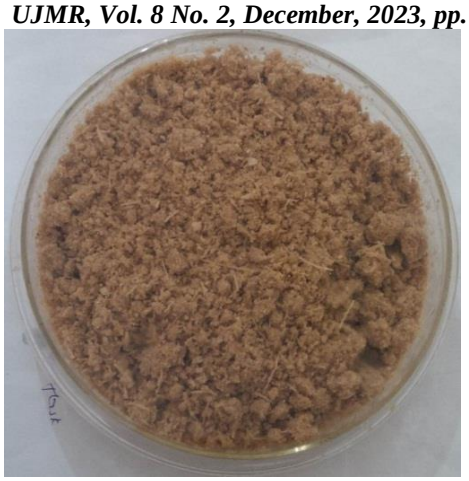

Plate I: Un-inoculated Sugarcane Bagasse (Control)

Plate II: *Aspergillus flavus* Inoculated on Sugarcane Bagasse

Plate III: *Trichophyton* sp. inoculated on Sugarcane Bagasse

Table 2: Amount of Sugarcane Bagasse Utilised by Fungal Isolates after 7days of Incubation at 25°C

Organisms	Initial Weight (W) (g)	Final Weight (W1) (g)	Amount consumed $(W - W1)$(g)	Utilised Proportion $\frac{(W-W1)}{W} \times 100$ (%)
SB + *Aspergillus flavus*	20	9.5	10.5	52.5
SB + *Trichophyton* species	20	13.5	6.5	32.5
Negative Control	20	20.0	0.0	0.0

KEY:
SB: Sugarcane Bagasse; W: Initial amount of bagasse (g) before incubation; W_1: Final amount of bagasse (g) after incubation

DISCUSSION

The compositional analyses of sugarcane bagasse had shown that cellulose (46.5%), hemicellulose (23.61%) and lignin (21.4%) were the most abundant fibre components. This is because generally, plant biomass, which is known as the most common source of Carbon, consists primarily of these three components (Badu *et al.*, 2011). Cellulose is seen to have the highest value, followed by hemicellulose and lignin respectively. These variations in composition of the crude fibre components of the sugarcane bagasse used in this study might be due to reasons such as differences in the varieties of sugarcane (Lacey, 1980) cultivated in various parts of the world (Kadam, 2000), portion of fibre on the cane of the sugarcane, cleanliness of the cane supplied, harvesting practices, as well as maturity of the sugarcane before it was harvested (Carvalho *et al.*, 2009; Irfan *et al.*,

2011; Zafar, 2015). The value of the sugarcane bagasse depends largely on its calorific value, which is also affected by its composition of sugars such as carbohydrates, glucose, galactose (Zafar, 2015) which constitutes most of the major nutrients needed for organisms to utilize for growth.

In most studies carried out which involved sugarcane bagasse, the proximate analysis revealed that cellulose has the highest percentage (about 35-60%), followed by hemicellulose (20-40%); and 10-30% lignin (Rodríguez-Chong *et al.*, 2004; Dawson and Boopathy, 2008; Carvalho *et al.*, 2009; Irfan *et al.*, 2011; De Souza *et al.*, 2012). Studies carried out by De Souza *et al.* (2012) showed that sugarcane bagasse consists of 48.6% cellulose, 31.1% hemicellulose, 19.1% lignin, with other components such as ash having 1.2%. In a report by Dawson and Boopathy (2008), proximate composition of sugarcane bagasse was said to consist of cellulose 30%; hemicellulose 23%; and 22% lignin. Irfan *et al.* (2011) also reported proximate composition of sugarcane bagasse as having about 40% cellulose; 23% lignin; ash and moisture content 0.9% and 7.1% respectively. The ash content generally has a low value, while the moisture content generally varies.

The growth of the fungal isolates *A. flavus* and *Trichophyton* sp. on sugarcane bagasse shows in each case, a pure culture of the organisms with no sign of any contaminant. This could be because sugarcane bagasse as a growth medium does not essentially meet the needs for growth of every microbe, most especially bacteria (Sidana and Farooq, 2014), and therefore, it can be used effectively to grow most fungi, as it minimizes culture media contamination by other microorganisms.

Fungi are also known to grow generally well on most organic materials ranging from plant, animal and human materials; and every fungal species require major elements namely Carbon, Nitrogen and energy source to grow and survive. Sugarcane bagasse as a residual material from sugarcane contains these materials in complex form such as carbohydrates, cellulose and lignin, which can be degraded by the fungi to grow and reproduce (Hasan *et al.*, 2015). Sidana and Farooq (2014) explained that sugarcane bagasse allows for growth of fungi with the production of more spores, as well as less bacterial contamination as is seen in other media, and observed that *Aspergillus* species had the maximum ability of spore formation than other fungi grown with the same media. Ojumu *et al.* (2003) reported sugarcane bagasse as a supporting medium for growth of A. *flavus*, while Bhattacharya *et al.*, (2011) also recorded A. *flavus* grown on sugarcane bagasse to be the best α-amylase producer in an investigation on the α- amylase production. Hasan *et al.* (2015) also reported a remarkable growth yield of Pink Oyster Mushrooms (*Pleurotus djamor*) using a supplement of sugarcane bagasse and wheat bran. A study carried out by Parani *et al.* (2016) in India had also shown 26.0%-60.3% degradation of sugarcane bagasse cellulose by Oyster Mushrooms. This is evident as other studies carried out with different species of the genus *Aspergillus* (e.g., A. *niger*), have shown its great efficiency to utilize sugarcane bagasse as substrate (Oliveira *et al.*, 2012).

However, there seems to be very scarce literature on the use of sugarcane bagasse to grow fungi belonging to the genus, *Trichophyton*. This study therefore provides evidence that *Trichophyton* sp. can also grow efficiently on sugarcane bagasse, as shown from the plates and table 2 above. Similarly, investigation of fungal growth on media formulated with sugarcane bagasse showed that several fungi from different genera, including *Trichophyton* species, could be efficiently grown on sugarcane bagasse substrate, based on their various degradative abilities of cellulose and other complex substances available in the substrate (Sidana and Farooq, 2014).

CONCLUSION

In conclusion, proximate composition of sugarcane bagasse carried out has shown that sugarcane bagasse consists mainly of carbohydrates, crude fibre, fat, protein, moisture and ash content. Cellulose, hemicellulose and lignin have also been found to be components of crude fibre.

Sugarcane bagasse inoculated with *Trichophyton* species and *Aspergillus flavus* showed that both organisms can utilise sugarcane bagasse as source of nutrient, with *Aspergillus flavus* exhibiting more growth (52.5%) than *Trichophyton* species (32.5%).

This study has also shown that sugarcane bagasse, which is one of the waste products found in Northern Nigeria could be converted into useful material, and as such, students of Microbiology could find easier ways of formulating media for growing some microorganisms during their researches.

RECOMMENDATIONS

It is recommended therefore that waste materials such as sugarcane bagasse can be easily used as alternative materials for producing laboratory media for easy cultivation of fungal isolates (without undergoing any form of industrial modifications specifically for isolation purposes (mostly for easy and more economical means of research for students) for growth of microorganisms especially fungi; as they are good sources of nutrients needed by

microbes. This could also serve as an easy and environmentally friendly means of ridding the environment from such waste materials.

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