The Effect of A. Fumigatus and A. Niger On the Sucrose Content of Saccharum Officinarum

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ABSTRACT

Sugar cane is an important crop with many industrial and domestic uses. Its constituents are useful in the production of alcohol, sugar or syrup, quality wax, livestock feed and as fuel. A total of twelve fungal strains belonging to three genera were isolated from three sugar cane stems and screened for *Aspergillus* sp. The identified *Aspergillus* sp. are *A. fumigatus* and *A. niger*. The two fungal strains were ascertained to be able to cause spoilage of sugar cane stems on the field and in storage by a simple pathogenicity test, they were both subjected to. The sucrose concentrations of the sugar cane stems employed reduced significantly, including the fibre and moisture contents. The fungal degradation on the stems recorded mean values of 15.06% sucrose, 10.26% crude fibre and 31.62% moisture content by *A. fumigatus* while *A. niger* recorded lesser values of 14.21% sucrose, 9.62% crude fibre and 27.56% moisture content respectively, after two healthy sugar cane stems were each deliberately inoculated with the fungal strains separately. A healthy sugar cane stem, which was used as the control, gave values of 31% sucrose, 14.5% crude fibre and 71.8% moisture content. The two strains were established to to reduce the nutritional content (Sucrose) of the sugar cane but with a greater effect from *A. fumigatus*. The propagation of resistant sugar cane species or cultivars and the use of appropriate fungicides on the fields should be adopted for better and optimal economic yield.

Keywords: Sugar cane, fungal degradation, pathogenicity, fungicides, resistant species

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1. INTRODUCTION

Sugar cane is a type of tall grass that is fertile in alluvial or red soil and is popular for cultivation worldwide for the production of sugar. It is used both as a cash crop as well as an alternative energy source. Among the various products sugar cane is used for, cane sugar (Table sugar) is the most common. Sugarcane or sugar cane are several species of tall perennial true grasses of the genus *Saccharum*, tribe Andropogoneae, native to the warm temperate to tropical regions of South, Southeast Asia, and New Guinea, and used for sugar production. The plant is two to six metres (six to twenty feet) tall. All sugar cane species can interbreed and the major commercial cultivars are complex hybrids (Del-bem, 2017). Sugarcane belongs to the grass family Poaceae, an economically important seed plant family that includes maize, wheat, rice, and sorghum, and many forage crops. *Saccharum officinarum* is a very important species of sugar cane, belonging to the family Graminae (Grasses) and to the sub-family Panicoideae.

Sugar cane is a large perennial tropical grass, cultivated for its tall, thick stems from which cane sugar, amounting to some 60 percent of the world's annual production of around 80 million tones of sucrose, is obtained (Cobley & Steele, 1996). The growth of sugar cane in the tropics is seldom limited by temperature. It tolerates occasional flooding and harvest begins when the sucrose content is acceptable. The length of the growing season for the plant-cane crop varies with climate. It may occupy the land for up to 24 months, although, the growing season for the harvested crop may only be 9 to 10 months (Fort & Smith, 2002).

The global demand for sugar is the primary driver of sugarcane agriculture. Cane accounts for 79% of sugar produced; most of the rest is made from sugar beets. Sugarcane predominantly grows in the tropical and subtropical regions (sugar beets grow in colder temperate regions). Other than sugar, products derived from sugarcane include falernum, molasses, rum, *cachaça* (a traditional spirit from Brazil), bagasse and ethanol. Brazil is the leading sugar producer in the world, followed by China and Thailand (Lichts, 2010). In Brazil, about half of the sugar cane crop is used for producing bioethanol.

Sugar cane juice is considered an alkaline-forming food because of the high concentration of calcium, magnesium, potassium, iron and manganese in it. As sugar cane juice boosts protein levels in the body, it helps in maintaining the health of the kidneys. Its alkaline nature can help to prevent diseases like cancer that cannot survive in an alkaline environment. Thus, it can helps in fighting various types of cancer such as prostate cancer and breast cancer. Its juice help to quickly hydrate the body, although, it contains a fair amount of calories which, if consumed in large quantities, can lead to weight gain, it should therefore be taken in moderate amounts, especially among the diabetics (Kamanzi,). Sugar cane juice is a diuretic, which means that it helps to treat urinary tract infection and kidney stones and it ensures proper functioning of the kidneys.

It can also be used to treat acidity and stomach burns. It is a good alternative to aerated drinks, which can be harmful to people with diabetes. The essential minerals it contains can help with digestion too. Sugar cane is an important crop with many industrial and domestic uses. The constituents are useful in the production of alcohol, sugar or syrup, quality wax, livestock feed and as fuel (Oduwobi, 2002). When sugar cane is produced for sugar cane manufacture, the crude by-products are bagasse, molasses and filter mud. In many parts of Nigeria, in particular, the Northern part, sugar cane stems are usually chewed to extract their sugary juice.

2. MATERIALS AND METHODS

Sample Collection

Part of the investigation was carried out in the Microbiology laboratory at The Federal Polytechnic, Ilaro, Ogun State, Nigeria. Healthy sugar cane stems were purchased from local vendors at Papalanto town, Ogun State. They were left for two weeks for them to naturally rot, for fungal isolation. Another healthy sugar cane stem was purchased thereafter but not allowed to deteriorate, to be used as the control.

Medium Preparation

The PDA used was prepared according to the manufacturer's instruction which is to dissolve 39g of dehydrated PDA in 1litre of distilled water and then autoclaved at 121°C for 15mins (Cheesebrough, 2000). The medium was allowed to cool to about 35^0C, an antibiotic (streptomycin) was incorporated to suppress bacterial contamination. Approximately 12ml of the molten PDA was aseptically dispensed into the sterile disposable 90mm Petri plates and then allowed to gel (Okigbo and Emeka, 1993).

Isolation

The method described by Gerhardt (1991) was employed to isolate fungi from the rotten sugar cane stem samples. Moistened sterile swab sticks were used directly on the infected portions of the samples by gently rubbing the sticks on the portions. The swab sticks were used to directly inoculate the already prepared PDA plates by direct streaking technique. The plates were incubated at $28^0C \pm 2^0C$ for four days.

Distinct colonies were sub-cultured for the fungal cultures to obtain axenic cultures by repeatedly streaking on fresh plates. The platted axenic cultures were then incubated appropriately. The axenic cultures isolated were preserved by streaking in McCartney bottles until the appearance of appreciable growth before they were then stored in a refrigerator. The pure cultures were kept refrigerated as stock cultures. The experiment was conducted in duplicate. Labeling of the sample containers was done accordingly.

Cultural Identification

The fungal isolates were identified on the basis of their morphological and cellular characteristics, as described by Barnet and Hunter (1999). The physical characteristics were compared to a Fungi Atlas for identification.

Pathogenicity Test

This test was conducted to determine the ability of the fungal isolates to cause disease on healthy sugar cane samples. To confirm the pathogenicity of the isolate, axenic cultures of the isolates were used to inoculate two separate healthy sugar cane stems. The samples were washed with distilled water and then surface-sterilized with 95% ethanol. A sterile scalpel was used to cut out a portion each of the samples for inoculation with the axenic cultures of the isolates onto the open cuts. The cuts were covered with sterile petroleum jelly at the points of inoculation. They were kept for two weeks. The localized appearance of signs and symptoms on the healthy samples confirm the pathogenicity of the isolates which could be identical to the diseased stems. This confirms Kosch's postulate (Okigbo *et al.,* 2009).

Sugar Cane Juice Extraction

The healthy (Control) and diseased sugar cane stems were each separately peeled, sliced and crushed using a warring blender, to extract the juice. The juice was obtained by filtration of the shaft from the mixture using a Muslin cloth, in turns. The filtrates were kept in labeled ErlenMeyer flasks for the subsequent analyses.

Proximate Analyses

The filtrates were analyzed for their moisture contents and crude fibre contents according to the methods of Allen *et al.* (1989) and Voogt (1978) respectively. The filtrate from the healthy sample was taken as the control, for comparative analysis. The mean values were recorded.

Sucrose Content Determination

The quantity of sucrose present in each filtrate was determined accordingly, using the procedure of the AOAC (1990), which include that of both the healthy and diseased samples. The mean values were recorded.

3. RESULTS AND DISCUSSION

Twelve fungal strains belonging to three genera were repeatedly isolated from three sugar cane stem samples and were screened for *Aspergillus* sp. The identified *Aspergillus* sp. were *A. fumigatus* and *A. niger.* The fungi isolated from the sugar cane samples, their fungal load, frequency and percentage of occurrence are shown in Table 1. Of these isolates, Isolate SS1 had the highest fungal load of 13.0 x 10^4 and with a percentage of occurrence of 41.70% while SS3 had the least fungal load of 7.0 x 10^4 and a percentage of occurrence of 16.66% respectively. Isolate SS2 had a fungal load of 10.0 x 10^4 and also a percentage of occurrence of 41.67%. Their frequency of occurrence ranged from 2 to 5.

Table 1: Fungal Load of the Isolates

Isolate code	Fungal load (cfu/ml)	Frequency of occurrence (n=12)	Percentage of occurrence (%)
SS1	13.0 x 10^4	5	41.67
SS2	10.0 x 10^4	5	41.67
SS3	7.0 x 10^4	2	16.66

Key: SS = Sugar Cane

The fungi isolated were identified based on their morphological and cellular characteristics: SS1 – *Aspergillus fumigatus*, SS2 – *Phytophthora infestans* and SS3 – *Aspergillus niger*. Table 2 shows the identities of the isolates.

Table 2: Fungal Strains Isolated From the Sugar Cane Samples

Isolate	Colonial appearance	Microscopy	Organism
SS2	Brown mold	Lumpy appearance, aplerotic	*Phytophthora infestans*
SS1	Filamentous black mold	Conidiophores tall and brownish, aseptate, mostly globose.	*Aspergillus fumigatus*
SS3	Black mold	Colonies spots jet black spherical conidia	*Aspergillus Niger*

Upon artificial inoculation onto healthy Sugar cane stem samples, the two *Aspergillus* strains produced characteristic symptoms on the inoculated samples the two weeks of the pathogenicity test. Table 3 shows the results of the proximate analyses and sucrose content of the diseased and healthy sugar cane samples.

Table 3: Comparative Analysis of the Proximate Analyses and Sucrose Content of the Sugar Cane Samples

Isolate	Sucrose	Sucrose	Mean	Moisture	Moisture	Mean	Fibre	Fibre	Mean
Aspergillus fumigatus	14.97	15.15	**15.06**	31.99	31.25	**31.62**	10.29	10.23	**10.26**
Aspergillus niger	13.92	14.5	**14.21**	28.14	26.98	**27.56**	9.87	9.37	**9.62**
Control (Healthy)	30.25	31.75	**31.0**	74.15	69.45	**71.80**	15.05	13.95	**14.50**

The sucrose concentrations of the sugar cane stems employed reduced significantly, including the fibre and moisture contents. The fungal degradation on the stems recorded mean values of 15.06% sucrose, 10.26% crude fibre and 31.62% moisture content by *A. fumigatus* while *A. niger* recorded lesser values of 14.21% sucrose, 9.62% crude fibre and 27.56% moisture content respectively, after two healthy sugar cane stems were each deliberately inoculated with the fungal strains separately. A healthy sugar cane stem, which was used as the control, gave values of 31% sucrose, 14.50% crude fibre and 71.80% moisture content.

The moisture and fibre contents of the sugar cane reduced significantly, just as the sucrose content, as the two fungal parasites established growth and infection on the sugar cane stems. However, *Aspergillus fumigatus* established a more significant deterioration than *Aspergillus niger*. The moisture content of sugar cane differs with variety and available soil moisture. Available soil moisture for the growth of sugar cane is simply expressed through the amount of juice that can be extracted. The reduction in the moisture and fibre contents in the diseased samples must have been due to the enzymatic actions i.e. degradation. Fungi, just like any other microorganisms, utilize water and nutrients for their physiological activities and cell constituents' make-up (Mead & Chen, 2007).

Sucrose is the most abundant carbohydrate in sugar cane. The glucose concentration is relatively high in immature stalks and decreases with maturation. It is most abundant in the regions of cell elongation, the tip of stalks and the base of young leaves. Although, hydrolysis of sucrose yields equal amounts of glucose and fructose, the glucose content is usually higher than the fructose content. Though, fructose occurs in the juice of cane stalks at about the same concentration as glucose but it is usually slightly less abundant than glucose (Fort & Smith, 2002). Fungi exhibit carbon heterotrophy and therefore obtain their carbon requirements from various organic sources. Fungi's utilization of starch takes place by enzymatic degradation but glucose and fructose are easily utilizable forms of carbohydrates being the most efficient source of carbon and energy for most fungi. The carbohydrates are utilized for physiological activities by fungi as well as for the synthesis of mycelia (Bilgrami & Verma, 1991). Fungi convert sucrose into glucose and fructose before assimilation.

4. CONCLUSION

From this study, the two fungal microbiota were established to have the potentiality to cause deterioration in sugar cane stems. It could also be deduced that *A. fumigatus* had a more severe impact on the sucrose concentration, moisture and crude fibre contents of the diseased sugar cane stems than *A. niger.* The healthy sugar cane (Control) had a relatively satisfactory result.

5. RECOMMENDATIONS

Fungal infestation can lead to huge economic losses and have tremendous adverse effect on the nutritional content of sugar cane. Sugar cane, with its numerous benefits, needs to be preserved and maximally utilized, hence:

- The propagation of resistant sugar cane species or cultivars should be promoted and encouraged.
- The use of appropriate fungicides on the fields should be adopted for better and optimal economic yield.
- Farmers should be educated and encouraged to propagate more of the resistant cultivars by Agricultural extension agents, to reduce or eliminate the chances of the spread of these fungal pathogens.
- Diseased stems could be processed into livestock feed or biofuel; turning waste to gain.

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