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Microbial Contamination, Field Diseases and Pests of Three Sugarcane Varieties Found in Port Harcourt

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Abstract

Study on survey of post harvest pathogen, field and storage pests and diseases of three varieties of sugarcane found in Port Harcourt was carried out in the Department of Plant Science and Biotechnology and Rivers State Teaching and Research Farm, all in the Rivers State University. Three sugar cane varieties identified based on stem colour viz: Kasha Cane-B51415 (Dark green), Anancy-BJ6183 (Green) and Kava Rangi-B64.277 (Red stem) were collected from Port Harcourt. The cultural laboratory technique was used for the microbial study while the hand book of South African Sugarcane Research Institute was used for field pests and diseases identification. Microbial shelf-life evaluation further revealed the red variety to contain reduced bacterial (3.8 $x10^6$, 3.5 $x10^6$ and 2.2$x10^6$ cfu/g) and fungal (0.7 $x10^4$, 1.3 $x10^4$ and 1.3 $x10^4$ cfu/g) loads for months 1, 2 and 3 respectively than other varieties within the storage months. A total of three bacterial organisms (Bacillus sp, Pseudomonas sp, Staphylococcus sp) and three fungal organisms (Aspergillus niger, A. flavus, Yeast) were isolated within the months of storage. Four field pests were observed viz: locust, aphid, trash caterpillar and fall army worm. However, none was recorded on the red variety. Five field diseases were observed including streak, red rot, rust, leaf spot and smut. The green variety recorded lowest number of prevailing field diseases. Generally, the three varieties of sugar cane performed differently with respect to pest and diseases.

Keywords: Sugarcane, pest, disease and survey

INTRODUCTION

Sacchurum holds its centre of origin in Asia, although it has been grown since 8000 BC (Fauconnier, 1993; Jackon, 2005; Aitken *et al.*, 2006). Notwithstanding, the crop is now grown widely both in the tropics and sub-tropic regions (Jangpronma *et al.*, 2010). Sugarcane is a perennial tall grass producing several stems. The plant is basically composed of leaves, stalk, root and inflorescence. The strong stem produces 30 to 60cm long linear green leaves. The stem which may vary in colour, can grow up to 5 meters in height (Riajaya *et al.*, 2022; DSPI, 2013).

The plant is mostly propagated by vegetative part (stem). Stem cuttings are planted few days after harvesting and germination takes place within two weeks after planting (Bull, 2000). Sugarcane

grows well in clay loam, sandy loam and clay soils and requires high rainfall and temperature (Blair and Stirling, 2007). In Nigeria, sugarcane is mostly grown in the Northern region and Nigeria ranks second for sugarcane production in West Africa (Sulaiman *et al.,* 2015). Sugarcane is an important crop with several economic benefits with sugar production being most important (OECD, 2011). Other by-products of sugarcane include ethanol, baggase, molasses, wax and ash (Hoareau *et al.,* 2006; Cheavegetti-Ganotto *et al.,* 2011).

Sugarcane is endowed with several nutrients including proximate, minerals and vitamins (Chuku and Emiri, 2018). The plant also possesses essential antinutrients (Wiiliams *et al.,* 2016). Literatures have also implicated the availability of these antinutrients in sugarcane to counter several medical properties to the plant including antioxidant; immunotherapeutic and anti-inflammation properties (Sepideh, 2016).

Sugarcane is affected by several diseases and pests. Insect pests have been reported to be associated with sugarcane and notable examples include borers, thrips, cane grubs, corn wireworm and spittle bugs (Kalunke *et al.,* 2009; Cherry, 2008). Vertebrate pests such as rodents, birds and pigs have also been reported to attack sugarcane (Serekebirhan, 2008 and 2011). Literatures have further reported numerous diseases (Scald, rust, smut, chlorotic streak, fiji leaf gall, yellow leaf virus and mosaic) caused by bacteria, fuji and bacteria to attack and damage sugarcane (Zhou, 2013; Zhao 2011; Ridley *et al.,* 2006; Gilbert *et al.,* 2009; Marcone, 2002).

The activities of these pests and diseases do not only affect the growth and yield but also the marketability and profitability for farmers and traders (Desalegn *et al.,* 2023; Paul and Huang, 2015).

MATERIALS AND METHODS

Sample Collection

Three sugarcane varieties (Red, Green and Dark Green), based on stem colouration according to Ekpelikpeze *et al.* (2016), were obtained from Port Harcourt and brought to the Department of Plant Science and Biotechnology, Rivers State University for proper identification by Dr. M. G. Ajuru, an Associate Professor of Plant Taxonomy and Systematics. The samples were also washed and preserved in refrigerator for microbial shelf-life assessment within three months.

Planting

The single bud direct planting method was adopted for this study (Desalegn *et al.,* 2023). Single bud sets of the sugarcane varieties were be planted directly into a spacing of 50cm x 50cm. A total plot area of 2.3 x 2m^2 was utilized for cultivation. The completely randomized block design (CRBD) was adopted for this study and each treatment replicated three times.

Field Pests and Diseases Survey Assessment

Observable signs and symptoms of disease and prevailing pests on the three varieties of sugarcane in the field were assessed with the aid of SASRI (2018) field guide. Pests were sampled in the morning and evening using sweep nets (Chuku *et al*., 2003).

Microbial Studies

Sterilization of conical flasks, slides, Petri dishes and all the equipment was carried out in the laboratory. The glasswares were sterilized in the oven at 120°C for an hour after washing with soap, while others equipment were surfaced sterilized with 70% ethanol to reduce Microbial contamination (Chuku, 2009). Inoculating loops and scapels were sterilized by dipping for 20 seconds in 70% ethanol and heated to red hot.

Preparation of Mycological Medium: Sabouraud Dextrose Agar was prepared in conical flasks using the standard method. The mouth of the flask was plugged with non absorbent cotton wool and wrapped with aluminum foil. The conical flasks containing the mycological Medium were autoclaved at 121°C and pressure of 1.1kg cm^{-3} for 15 minutes. The molten agar was allowed to cool to about 40°C and dispensed into Petri dishes at 15mls per plate and allowed to further cool and solidify.

Isolation: A threefold serial dilution (10^{-3}) was used in accordance to the method of Mehrotra & Aggarwal, (2003) where 1g of the spoilt sugarcane fruit samples were transferred into the first test tube containing 9mls of normal saline. 1ml of the solution was transferred to the second test tube and finally from the second to the third. 0.1ml aliquot from the second and third dilution was plated onto Nutrient Agar in Petri Dishes and this was done in triplicate. The inoculated plates were incubated for 5 days at ambient temperature of 25C ± 3°C (Chuku, 2009). The entire set up was observed for 7days to ensure full grown organisms. Pure cultures of isolates were obtained after a series of isolation (Obire *et al.*, 2016).

Characterization and Identification of Bacteria

Identification of bacterial isolates was based on their cultural morphology, microscopic examination and biochemical test. Morphological studies were carried out on different media plates used for the Isolation of the organisms; pure colonies were isolated based on colony size, shape, pigmentation, elevation and texture of the organism after 48 hours of growth at 30°C. Pure isolates from the respective media were characterized and identified based on their morphological, biochemical and physiological features (Cheesbrough, 2000).

Biochemical Tests: which include catalase, indole production, methyl red, vogues proskauer (MRVP) and sugar fermentation test was carried out according to the method of Cheesbrough, 2000.

Identification of fungi

Microscopic examination of fungal isolates was carried out by the needle mount method (Cheesebrough, 2000). The fungal spores were properly teased apart to ensure proper visibility. The well spread spores were stained with cotton blue-in-lacto phenol and examined microscopically using both the low and high power objective. The fungi were identified based on their spore and colonial morphology, mycelia structure and other associated structures using the keys of (Barnett and Hunter, 1998).

RESULTS AND DISCUSSION

Table 1: Microbial Occurrence on Stored Sugarcane Varieties

Varieties	Bacteria isolates	Fungi isolates
Red Stem	*Bacillus* sp., *Staphylococcus sp*.	*Aspergillus niger*, *A. flavus*, yeast.
Green stem	*Bacillus* sp., *Pseudomonas sp*., *Staphylococcus sp*.	*Aspergillus niger*, *A. flavus*, yeast.
Dark green stem	*Bacillus* sp., *Staphylococcus sp*.	*Aspergillus niger*, yeast.

Table 2: Microbial Enumeration of Stored Sugarcane Varieties

Varieties	THB (10^{-6})	THF (10^{-4})
		Month 1
Red Stem	3.8 x10^{6}	0.7 x10^{4}
Green stem	7.8 x10^{6}	1.1 x10^{4}
Dark green stem	4.5 x10^{6}	0.7 x10^{4}
		Month 2
Red Stem	3.5 x10^{6}	1.3 x10^{4}
Green stem	6.0 x10^{6}	1.7 x10^{4}
Dark green stem	4.8 x10^{6}	0.9 x10^{4}

		Month 3
Red Stem	$2.2x10^6$	$1.3 x10^4$
Green stem	$5.1x10^6$	$2.9 x10^4$
Dark green stem	$2.6x10^6$	$2.3 x10^4$

Total Heterotrophic Bacteria (THB), Total Heterotrophic Fungi (THF)

Table 3: Survey of Field Diseases of three Cultivated Sugarcane Varieties

Diseases	Green	Dark green	Red cane
Maize streak	-	+	-
Red rot	+	+	+
Rust	+	+	+
Leaf spot	+	+	+
Smut (Die back)	-	-	+

Present (+), Absent (-)

Table 4: Survey of Field Pests of three Cultivated Sugarcane Varieties

Pest	Green	Dark green	Red cane
Locust	-	+	-
Aphid	-	+	-
Trash caterpillar	+	-	-

Fall army worm	**+**	**-**	**-**

Present (+), Absent (-)

Within the storage period of three months, the sugar cane varieties were also assessed for microbial load. A total of three bacterial genera (*Bacillus, Staphylococcus* sp and *Pseudomonas* sp.) and three fungal organisms (*Aspergillus niger, A. flavus* and *Yeast*) were isolated within the storage month (Table 1). The red variety recorded the presence of *Bacillus, Staphylococcus, A. niger, A. flavus* and *Yeast.* Dark green variety was associated with *Bacillus, Staphylococcus, A. niger* and *Yeast.* All isolated bacterial and fungi were seen in the green variety.

The monthly microbial enumeration presented in Table 2 showed highest bacterial counts (7.8×10^6, 6.0×10^6 and 5.1×10^6) for months 1, 2 and 3 respectively for the green variety. However, the red variety recorded lowest bacterial counts (3.8×10^6, 3.5×10^6 and 2.2×10^6) for months 1, 2, and 3 respectively. The green variety also record highest fungal counts (1.1×10^4, 17×10^4 and 2.9×10^4) for months 1, 2 and 3 respectively.

On the contrary, lowest fungal count (0.7×10^4) was observed for both the red and dark green variety for month 1. Furthermore, the lowest fungal count at month 2 (0.9×10^4) and month 3 (1.3×10^4) were recorded for the dark green and red varieties respectively. Generally, a contrasting progress in microbial count was observed for the fungal and bacterial isolates as the fungal count increased when the month increased while bacterial count decreased as the months increased. The green variety had more isolates and counts than every other variety while the red variety performed best with the lowest microbial count.

The present study further profiled the associated bacterial and fungal organisms associated with stored sugarcane stems. Microflora observed include *Bacillus* sp., *Staphylococcus* sp., *Pseudomonas* sp., *Aspergillus niger*, *A. flavus* and Yeast. The fungal isolates are in line with the report of Younes and Embaby (2024) as they also reported *Aspergillus* sp., *Fusarium, Penicillium, Rhizopus* sp., *Trichoderma* sp. and *Alternaria* sp. to be associated with harvested sugarcane stem. They further reported the ability of *Aspergillus* sp. to produce deadly mycotoxins that are detrimental to humans when ingested.

The current study considered the microflora of all three sugarcane varieties stored in refrigerator for three months. It was observed that the red variety had lesser microbial load (fungi and bacteria) while the green variety had highest microbial load. The availability of high concentrations of phytochemical in the red variety could be the possible reason for the reduced microbial load as literatures have implicated antimicrobial activities of these chemicals on bacterial and fungal organisms (Singh *et al.*, 2015).

In addition, the microbial count for the storage duration revealed higher bacterial population than the fungi for the three months. More so, it showed a decrease in the bacterial population as the month decreased and an increase in the fungi population as the month increased. This could be related to the fast generation time of bacteria than fungal organisms as fungal organisms are known to grow slower than bacteria (Mehrotra and Aggarwal, 2003).

On the other hand, the decreased population of bacteria as the month progressed could also be as a result of the antibiotic produced by the emerging fungal organisms in the stored sugarcane samples (Mehta and Ayoub, 2024).
Notwithstanding, there is dearth of information on the microbial contamination of sugarcane stem, as several literatures are focused on the microbial spoilage of sugarcane products such as confectionaries and juice (Ranaware *et al.*, 2020; Tha *et al.*, 2023; Rana *et al.*, 2024). Different means of sugarcane product preservation other than refrigeration has also been reported (Sujatha *et al.*, 2023).

Field diseases and pests were observed among the cultivated varieties. The result of field disease survey presented in Table 3 revealed a total of five diseases (streak red rot, rust, leaf spot and smut in the experimental field.
All diseases were seen on the dark green variety with an exception for smut while the red variety recorded every other disease but streak. The green variety recorded lowest number of disease as it had only red rot, leaf spot and rust.
The result of field pest survey presented in Table 4 showed the occurrence of four pests on the experimental field viz: Locus, Aphid, Caterpillar and army worm.
The green variety recorded the presence of the trash caterpillar and fall army worm while the dark green had the locust and aphid. No pest was observed for the red variety.

The current study has revealed the various pests and diseases associated with the green, red and dark green varieties of sugarcane within the study are Port Harcourt. however, streak, red rot, rust, leaf spot and snout were the only diseased recorded. Notable pests were also observed including locust, aphid, trash caterpillar and fall army worm. Although, most of these pests was associated with the red variety. Literatures have shown the menace of pests, diseases and weeds on the cultivation of sugarcane as it does not affect yield and yield components but eventually lead to poor marketability and profit (SRA, 2023).

Ajayi *et al.* (2020) also indicated the variation in pest and disease susceptibility by sugarcane varieties. They revealed the occurrence of red rot, mosaic and termite on cultivated sugarcane. The pest data of the current study agrees with the report of Raut *et al.* (2024) as they recorded grasshopper, aphid, caterpillar and army worm to be associated with sugarcane in the field. Similar situation was also reported by Kumar *et al.* (2019) for sugarcane pest. The diseases recorded in the current study have been associated with pathogenic fungal organisms (Usman *et al.*, 2020). The reduced pest on the red variety could be as a result of the high phytochemical components.

CONCLUSION

All utilized sugar cane variety performed differently in the assessed experiment. However, the red variety had no associated field pest and reduced post harvest pathogen within the duration of study. The dark green variety showed high level of growth parameters (plant height, number of leaves and length of leaves) and less field diseases susceptibility than other varieties. The green variety performed poorly with respect to the variables assessed with an exception of field disease.

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