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Effect of Red Rot Pathogen (*Colletotrichum falcatum*) on the Nutritional Contents of Sugarcane in the Savannah Ecological Zone of Nigeria

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ABSTRACT

Sugarcane production in Nigeria faces significant challenges, with red rot disease caused by *Colletotrichum falcatum* being a major concern. This study aimed to assess the effect of red rot pathogen (*Colletotrichum falcatum*) on the nutritional contents of sugarcane in the Savannah Ecological Zone of Nigeria. The fungal pathogen was successfully isolated and identified from diseased sugarcane plants, and its pathogenicity was confirmed through induced red rot symptoms on sugarcane plants. All experiments were conducted using standard procedures and data were analyzed using Analysis of Variance (ANOVA) at 5% probability level of significance. Results of the proximate composition analysis highlighted differences in nutritional content between uninfected and infected sugarcane, with infected samples exhibiting higher moisture of 15.85%, ash, 6.36% and fiber content 1.56%, against 6.68%, 6.30% and 1.24% of uninfected samples. While the uninfected samples having higher protein 1.72% and carbohydrate content 84.06% as against 1.11% and 79.59% of infected samples. Although proximate analysis results showed differences in the nutritional contents of the infected and uninfected sugarcanes, analysis of variance show significant difference between infected and uninfected samples in terms of carbohydrates and proteins contents, however, no significant difference existed between the two samples in terms of ash and fibre contents. Overall, it is clear from the result that the fungus not only cause red rot on sugarcane plants but also reduces the carbohydrates and proteins contains of sugarcane and by extension quality of sugar as well which might have a remarkable effect on the value of the sugar. It is therefore recommended timely spraying of the cane with fungicides to reduce the damaging activities of the pathogen and contamination with mycotoxins and other related fungal metabolites that might be harzadous to human health.

Keywords: *Colletotrichum falcatum*, Nutritional contents, Red rot, Sugarcane

INTRODUCTION

Sugarcane (*Saccharum officinarum*), belonging to the Poaceae family, is a perennial grass widely cultivated for its juice, which serves as a primary source for sugar production. Additionally, sugarcane is utilized for ethanol and biofuel production, making it an essential cash crop in tropical and subtropical regions worldwide (Dacosta *et al*., 2011). With its rich sucrose content, sugarcane accounts for a significant portion of global sugar production, making it a vital commodity in the agricultural sector (Menossi *et al*., 2008). As of 2008, global sugarcane production reached 1.5 billion tons, with leading producers including Brazil, China, Cuba, Mexico, Pakistan, Thailand, USA, Colombia, Australia, and Indonesia. Africa, during the same period, contributed 1.2 million hectares to sugarcane cultivation, yielding 72.1 million metric tons. Nigeria, boasting a potential land area of over 500,000 hectares for sugarcane cultivation, holds a prominent position among sugarcane producers, with the capacity to yield approximately 3.0 million metric tons of sugar if processed (Imolehin and Wada, 2008).

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Red rot disease, caused by *Colletotrichum falcatum*, poses a significant threat to sugarcane production globally (Viswanathan and Samiyappan, 2002; Viswanathan, 2011). This longstanding and widespread disease affects sugarcane plants from the seedling stage to maturity, often leading to discoloration, reduced cane weight, and losses in sugar, sucrose content, and juice (Sharma and Tanta, 2015; Wada *et al*., 2016). In Nigeria, surveys have identified red rot disease in states such as Niger, Katsina, Benue, Plateau, Adamawa, and the FCT, Abuja (Wada *et al*., 2016), underscoring its impact on sugarcane cultivation in the country. The economic repercussions of red rot disease are substantial, with the sugar industry experiencing annual losses exceeding 500 million dollars due to reductions in sucrose content and cane weight (Viswanathan and Samiyappan, 2002). This disease not only affects global export sectors but also jeopardizes domestic production, as many locally preferred sugarcane cultivars are at risk (Sharma and Tanta, 2015; Wada *et al*., 2016).

In view of the above problems and need to often data specific to savannah region of Nigeria where sugarcane production is prominent, this research aimed to study the effect of red rot pathogen on the nutritional contents of sugarcane.

MATERIALS AND METHODS

Study Area

The research was conducted at the laboratory of the Department of Plant Science, Modibbo Adama University, situated in Yola, Nigeria. Modibbo Adama University, located in Girei Local Government Area, falls within the Northern Guinea Savannah Zone of Nigeria, located on latitude 9°18'00"N to 9°21'40"N and the longitude of 12°28'30"E of the Greenwich Meridian. This region experiences arid and semi-arid climates, characterized by distinct dry and rainy seasons. The dry season typically spans from late November to May, accompanied by the influence of the harmattan wind originating from the Sahara Desert (Adebayo, 2020). Monthly mean temperatures during the dry season range between 31°C to 40°C for maximum temperatures and 15°C to 23°C for minimum temperatures. April typically registers the highest temperatures, representing the peak of the dry season, while December and January record the lowest mean monthly temperatures, corresponding to the winter period in the region (Geography Department, 2021). The climate data utilized in this study were obtained from the Geography Department of Modibbo Adama University, Yola.

Sample Collection

Infected and healthy sugarcane stalks and setts were gathered from a sugarcane farms located at Dangote Sugar Refinery in Numan, Adamawa State. The samples were kept in the clean polythene bags sealed and transported to the laboratory of the Department of Plant Science in Modibbo Adama University, Yola for further studies.

Experimental Design

Completely randomized design (CRD) was adopted for this study. Each treatment was replicated five times.

Preparation of Potato Dextrose Agar (PDA)

Potato Dextrose Agar (PDA) were used in isolating the fungi. The medium was prepared by suspending 39 g of the powder in 1000 ml of distilled water (as for manufacturer instruction). This was mix while boiling to dissolve the suspension completely. It was then sterile by autoclaving it at the temperature of 121^0C for 15 minutes. The medium was then poured into a sterile petri dish under sterile conditions and allowed to solidify according to Adebola *et al*. (2016).

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Isolation and Identification of Fungal Pathogen

Isolation was conducted according to Adebola and Amadi (2010); Zakari *et al*. (2016). The infected sugarcane stalk was cut open into 1.0 - 1.5 cm segments using sterile blades with middle transition zone of healthy and infected portion. The samples were washed thoroughly under running tap water, surface sterilized using 0.5 % sodium hypochlorite for 30 to 40 seconds and washed five (5) times with sterile distilled water. The surface sterilized sample segments were inoculated on Petri dish containing Potato Dextrose Agar (PDA) at room temperature 28±2 °C in an incubating chamber. Subcultures will be done to obtain pure culture of the pathogen. The pathogen was identified using the cultural characteristics like colony color and morphological characteristics such as the shape and size of macro and micro conidia under the microscope using manuals of soil fungi (Adebola and Amadi, 2010). Stock culture of the isolate was maintained in McCartney bottle slants and stored at 4 °C in refrigerator for subsequent use (Adebola and Amadi, 2010).

Preparation of Inoculum Suspension

Inoculum suspension was prepared by inserting 5 mm of culture medium with fungus in the Centre of the Petri dishes containing Potato Dextrose Agar (PDA). The inoculated plate was transferred in to the incubation chamber until the fungus filled the plate. After sporulation, conidia were collected and added to 10 ml of sterile distilled water, rubbing a brush slightly over the colonies and subjected the suspension to constant agitation until the spores get liberate. This was used to determine the concentration of conidia suspension (Fernando, 2013). The macro and micro conidia were adjusted to a concentration of 10^6 conidia /mL suspension.

Pathogenicity Test

To ascertain the pathogenicity of the organism, apparently healthy stalk of growing Sugarcane (three stands) were planted inside seven (7) liters polythene bags containing sterilized soil. The plants stalks were inoculated with 2 mL spore suspension of *C. falcatum* using a 2 mL syringe and needle, while the control pots were inoculated with sterile distilled water without conidia suspension and were observed for disease symptoms. *C. falcatum* was re-isolated from the inoculated plants showing the symptom of the disease, confirming it pathogenicity.

Proximate Analysis

Proximate analysis comprising moisture, ash, crude fat, crude proteins, and carbohydrates contents was determined by their respective formulation of raw material using standard procedures as described below;

Sample preparation

The healthy and diseased sugarcane stalks were purchased from Geire market, Adamawa State, Nigeria, and dry-grounded into powder, weighing 900 g, and packaged in glass. The healthy and diseased sugarcane were extracted three times by repeated boiling from matured organic sugarcane without sulfur treatment.

Moisture content

The moisture content of both healthy and diseased sugarcane was analyzed using the drying method. Five grams of healthy and diseased sugarcane were dried in a conventional oven (Thermo Fisher Scientific, U.S.) at 105°C for 24 hours. Then, the sample was cooled in a desiccator for 20 minutes and weighed to calculate the percentage weight loss of the sample using formula:

Moisture (%) = (Weight of sample used – Dry weight of sample) × 100.

Weight of sample used

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Ash contents

The ash content of healthy and diseased sugarcane was determined using the dry ashing method. Five grams of healthy and diseased sugarcane samples were placed into the Muffle furnace (Carbolite, England) at 550°C for approximately 12 hours until they turned to ash. The ash content was determined using formula:

$$\text{Ash (\%)} = \frac{\text{(Weight of +weight of crucibles) - (Weight of crucible)}}{\text{Weight of sample}} \times 100$$

Crude fat

The analysis of crude fat in healthy and diseased sugarcane was carried out using the Soxhlet method. Five grams of the sample were transferred into a Soxhlet extraction flask (Fat Extractor E-500). Next, 200 ml of petroleum ether (Merck, Germany) was poured into the boiling flask attached to the Soxhlet extraction flask. The apparatus was heated in a boiling water bath to reflux the solvent for 8 hours. Then, the sample was cooled down by rotary evaporation. The flask was then placed in a conventional oven for 15 minutes at 105°C. Lastly, a desiccator was used to cool down the flask containing the sample again, and it was weighed. The crude fat was measured using formula:

$$\text{Fat (\%)} = \frac{\text{Weight of oil}}{\text{Weight of sample}} \times 100$$

Crude protein

For crude protein content, the micro-Kjeldahl method (method 950.36) was used. 0.15 g of healthy and diseased sugarcane shifted in a boiling tube. Then, 0.8 g of mixed catalysts and 2.5 ml of concentrated sulfuric acid (H_2SO4) (Merck Germany) were added to the tube and heated. Next, 10 ml of 45 % sodium hydroxide (NaOH) solution (Merck, Germany) were slowly added to the distillation tube to separate the two layers of the solution. The conical flask containing 2% boric acid (Merck Germany) was placed on the distillate platform, and the distillation of ammonia was allowed to take place. The ammonium borate in the distillate was titrated with 0.05 N H2SO4 until the end point was reached, and the amount of titrating was recorded. The percentage of proteins was calculated using formula:

$$\text{Protein (\%)} = \frac{\text{(The volume of titrating sample – Volume of titrating blank)} \times 0.05N \times 14 \times 100}{\text{Weight of sample}}$$

Crude fibre content

The crude fibre content was determined using method 962.09 of AOAC. About 0.5 g of the sample was boiled in 50 ml of 0.3 M H_2SO4 under reflux for 30 minutes, followed by filtering through a 75 mm sieve under suction pressure. The residue was washed with distilled water to remove the acid. The residue was then boiled in 100 ml of 0, 25 M sodium hydroxide under reflux for 30 minutes and filtered under suction. The insoluble portion was washed with hot distilled water to free the alkaline. The insoluble portion was dried to a constant weight in the oven at 100 °C for 2 hours, and then cooled in the desiccator. The dried sample was ashed in a muffle furnace to subtract the mass of ash from the fiber, and then the percentage of fibre was determined.

Carbohydrates

The total carbohydrates content of healthy and disease sugarcane was determined by difference using formula:

% total carbohydrate = 100 – (% moisture + % ash + % crude fat + % crude proteins + % fibre).

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Data Analysis

Data obtained from the proximate analysis were subjected to statistical analysis of variance (ANOVA) to determine the significant differences among means. The Least Significance Different (LSD) was used to separate the means where there are significant differences. The analysis was carried out using the Paleontological Statistics, at a 5% level of significance.

RESULTS

Isolation and Identification of the Fungal Pathogen

The fungal pathogen of red rot of sugarcane was isolated from diseased sugarcane plants collected from different sugarcane fields in the Dangote Sugar Refinary, Numan, Adamawa State, Nigeria. The fungal isolate was identified according to its characteristics appearance on culture media under a light microscope (Table 1 and Plates I A and I B).

Pathogenicity test

The result of the pathogenicity test showed that the fungal isolate was pathogenic on sugarcane plants after fifteen (15) days of inoculation. The development of the red rot symptom was rapid, leading to the reddish rotting of the plant tissues (Plates II and III). There was no symptom development on sugarcane setts inoculated with sterile distilled water. The same fungus was re-isolated from the diseased plant tissue.

Comparative Proximate Composition the Healthy and Diseased Sugarcanes

Proximate compositions of the fungal-infected and healthy sugarcanes were presented in Table 2. Results indicated that there were increase in moisture (15.85 %), ash (6.36 %), and fibre (1.56 %) content of the fungal infected canes compare to the healthy ones with 6.68 %, 6.30 %, and 1.24 % moisture, ash, and fibre contents respectively, while the proteins (1.72 %), and carbohydrates (84.06 %) contents of the healthy canes were higher than those of the fungal infected canes with 1.11 %, and 75.12 % proteins, and carbohydrates contents respectively.

Table 1: Characteristics of *Colletotrichum falcatum* isolated from Sugarcane in Savannah

Fungal pathogen	Cultural characteristics	Morphological characteristics
Colletotrichum falcatum	Mycelia light loose, cottony, and almost white in young cultures, becoming light ashy with broad white magins in 7 to 14 days	Conidia were curve or falcate shape with hyaline having two septate.

Table 2: Proximate composition of fungal infected and healthy sugarcanes

	Percentage Composition				
Nutritional contents	**Moisture**	**Ash**	**Protein**	**Carbohydrate**	**Fiber**
Healthy Sugarcane	6.68	6.30	1.72	84.06	1.24
Diseased Sugarcane	15.85	6.36	1.11	75.12	1.56
Mean	11.27	6.33	1.42	79.59	1.40
p-value	0.00019	0.9557	0.03571	0.001982	0.5729
LSD	0.67	1.22	0.22	1.41	0.58

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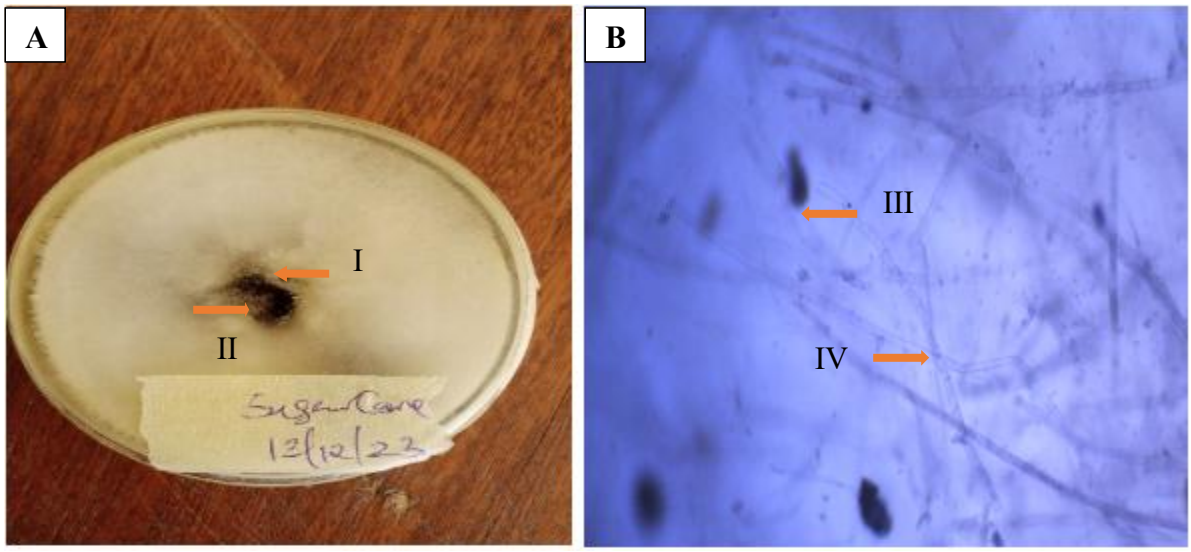

Plate I: Cultural and morphological characteristics of a seven-day-old *C. falcatum* (A-front plate, I & II- black old and white advancing mycelia, B-Conidia at x10 objective, III & IV- conidium and conidiophore).

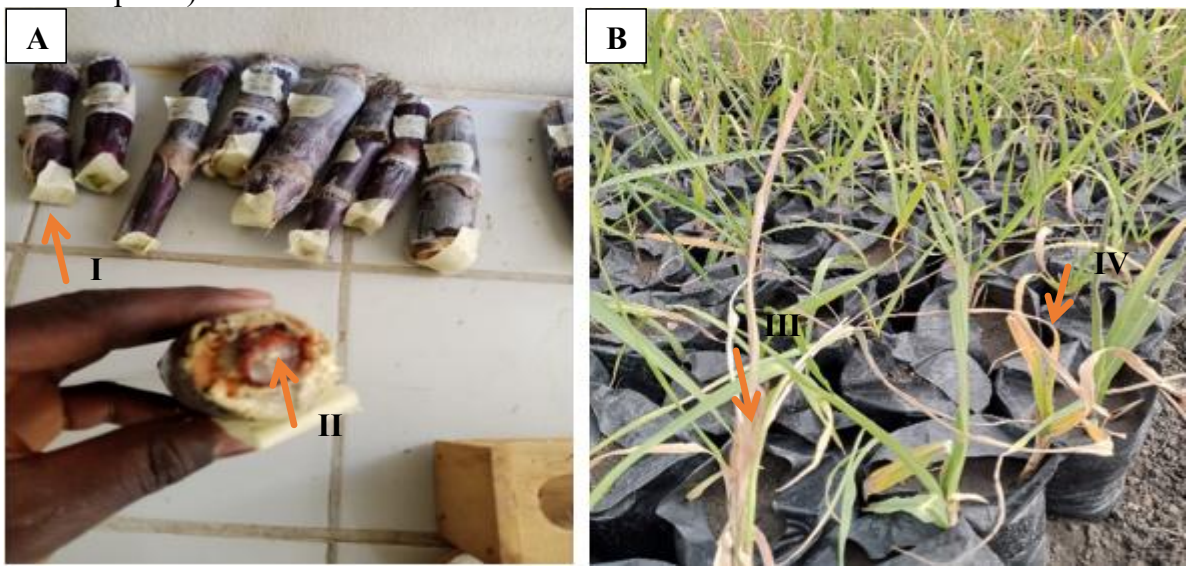

Plate II: Pathogenicity test of *C. falcatum* (A-inoculated setts, I &II- sealed inoculated portion and red rotted tissue at seven days after inoculation, B-inoculated sugarcane seedlings, III & IV- brownish leaf of infected seedling and Pot containing infected soil).

DISCUSSION

Colletotrichum falcatum was the fungi isolated from diseased sugarcane samples collected from fields across the sugarcane cultivation sites in Dangote Sugar Refinary Numan. This fungus was also reported to have been isolated from sugarcane in different parts of the world (Viswanathan and Samiyappan, 2002; Sharma and Tanta, 2015; Wada *et al*., 2016). Pathogenicity of the fungal isolate was conducted on sugarcane setts and on potted sugarcane seedlings under laboratory and field conditions. The isolate was pathogenic on the sugarcane tissues examined.

The variation in the nutritional contents of the fungal-infected and apparently healthy

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sugarcanes was explained by several scientists across the world. Rott (200) reported that red rot-infected sugarcane often exhibits higher moisture content compared to healthy sugarcane that can be attributed to the metabolic activity associated with the infection process and water uptake by the plant tissues. Ash content, representing the inorganic mineral content, may vary between healthy and infected sugarcane. These alterations in ash content in diseased sugarcane may be due to changes in nutrient uptake and metabolism (Ravichandra *et al*., 2015). The diversion of nutrients towards defense mechanisms against the pathogen may result in reduced protein synthesis (Ghosh *et al*., 2017).

Crude fat content in sugarcane is generally minimal, but red rot infection can influence lipid metabolism in the plant. These changes in crude fat content may reflect alterations in metabolic pathways induced by the disease (Vidhyasekaran *et al*., 2014). Infected sugarcane may exhibit alterations in crude fiber content due to structural modifications induced by red rot infection. Increased crude fiber levels can indicate cell wall thickening or lignification as a response to pathogen attack (Ravichandra *et al*., 2015). Carbohydrate metabolism is significantly affected by red rot infection, leading to changes in carbohydrate composition and content. Reduced carbohydrate levels in infected sugarcane may result from disrupted photosynthetic activity and nutrient translocation (Vidhyasekaran *et al*., 2014.

CONCLUSION

It may be concluded from this study that *Colletotrichum falcatum* is a common pathogenic fungi which cause red rot disease of sugarcane in the study area. The result from the pathogenicity test indicated that the isolated fungus is pathogenic and attributed to the cause of red rot disease of sugarcane in the region. It is also clear from the result that the fungus not only cause red rot on sugarcane plants but also reduces the nutritional values of sugarcane and by extension quality of sugar as well which might have a remarkable effect on the value of the sugar. It is therefore recommended timely spraying of the cane with fungicides to reduce the damaging activities of the pathogen and contamination with mycotoxins and other related fungal metabolites that might be hazardous to human health.

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