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Abstract. The study was carried out to determine the effect of fungal rot disease on nutritional value of banana fruits. Standard methods were used to carry out the study. Results of this study show the presence of Colletotrichum musae, Aspergillus niger, Aspergillus flavus and Rhizopus stolonifer on the rotten banana fruits and result of proximate analysis of the diseased banana fruits shows higher moisture content (75.85%) than the apparently healthy banana fruits (74.34%). There was decrease in the crude protein (2.63%), carbohydrate (19.54%), lipid extract (0.20%), ash (0.91%) and crude fibre (0.88%) contents of the rotten fruits relative to the apparently healthy ones. Vitamin B2 (riboflavin), vitamin B9 (folic acid) and vitamin C values are significantly low in infected banana fruits relative to the values of apparently healthy ones. The results are clear evidence that C. musae, A. niger, A. flavus and R. stolonifer bring about reduction in the nutritional value of banana fruits, therefore, banana should be carefully handled to preserve their nutrient composition. This study has shown that banana fruits contain high level of vitamin B<sub>9</sub> with low lipid and fibre; this will help the general public especially, fruitarians and dietitians to make proper combination of their foods.

Key words: Banana; effect; fruits; fungi; infection; nutritional value.

### **INTRODUCTION**

Bananas which belong to a group of fruits known as berry are edible fruits [16]. They are in the genus and family Musa and Musaceae respectively [10]. The plant has succulent, very juicy pseudostem which is a cylinder of leaf-petiole sheaths, reaching a height of 6 -7.5 m and arising from a fleshy corm [10]. Banana fruits vary in size, colour and firmness, but is usually elongated and curved, with soft flesh rich in starch covered with a rind which may be green, yellow, red, purple or brown when ripe [9]. The fruits grow in clusters, hanging from the top of the plant. Almost all modern edible parthenocarpic bananas come from two wild species namely Musa acuminata and Musa balbisana [7].

Banana fruits are staple fruits in many countries of the world because they are good source of potassium, vitamins and other nutrients which protects against some chronic diseases like diabetes and cancers [25]. They are relatively low in calories but rich in vitamins [14]. According to Robson [21], children who eat bananas everyday have up to 34 % reduced risk of displaying asthma symptoms. This is due to bananas rich vitamin B<sub>6</sub> content, also known as pyrixodine, which aid in relaxing bronchial muscle tissue. Bananas are also rich in powerful antioxidants like catechins and dopamine which are associated with numerous health benefits [17]. Potassium, an essential mineral for cardiovascular health and blood pressure control, is abundant in banana fruits, making them an ideal dietary source [22]. Recently, pounded banana peels have been found to contain antibiotic properties and are used in many pacific cultures as wound bandage [17]. Banana fruits are always susceptible to a fungal attack when in storage and transit and the result of such attack

is reduction in the market and nutritional value of the fruits [24].

Banana fruits infected by fungi always lose market and nutritional values; among the diseases are crown rot, anthracnose and cigar-end rot [3]. Crown rot is one of the most postharvest diseases of bananas [1]. It is characteristically complex phyto-disease caused by several fungi in synergy with other micro-organisms such as bacteria [6]. The most common pathogens associated with crown rot are Colletotrichum musae, Gloesporium musarum Fusarium roseum, Fusarium semitectum and Lasiodiplodia theobromae [4]. Anthracnose is another postharvest disease of banana, which develops during the storage and ripening of the banana [12]. Colletotrichum musae is the most common species associated with anthracnose of banana and has been widely accepted as the causal agent of the disease [13].

Although, banana fruits in our daily food requirements cannot be over-emphasized, there is paucity of information on the effect of fungal infection on their nutritional value; even the available report like Subash and Saxena [24]. This study aimed to determine the effects of fungal rot on banana (Musa sapientum) fruits in Abakaliki Metropolis, Ebonyi State, South-eastern Nigeria

### MATERIALS AND METHODS

#### Study area

The study was carried out in Abakaliki metropolis, Ebonyi State, South-eastern Nigeria.

#### Sample collection and preparation

A total of forty fingers of banana fruits (both infected and apparently healthy ones) were randomly purchased from Ahia-ohu market in Abakaliki metropolis of Ebonyi State. All the banana fruits purchased were allowed to stay in a sterile incubator at room temperature for 24 hours to clearly ascertain the infected ones.

### Isolation of the fungi

Fungi associated with the infected banana (*Musa sapientum* L.) fruits were isolated using the blotter method as recommended by the International Seed Health Testing Association [8]. Portion of the rotten banana fruits were sliced into smaller portions, washed with clean water, surface sterilized with 0.01% mercuric chloride for about 30 seconds. These were then rinsed in sterile distilled water and inoculated on solidified potato dextrose agar medium (PDA). The plates were incubated at room temperature  $(25\pm2^{\circ}C)$  for 24 hours. There was repetition of sub-cultures obtained from fungi that grew from the incubated plates until pure cultures were obtained [28].

### Identification of the fungi associated with infection

Identification of the fungi associated with the infection was done by studying the characteristics of the fungi on the culture media. Their morphological structures were also observed under the light microscope with  $\times 400$  magnification. These structures were then compared with the structures in the fungi identification manual [26, 28].

## Pathogenicity test

Pathogenicity of the fungi isolated was determined using the approach of SPT-TCA [23]. Apparently healthy banana fruits were washed with clean water and then surface sterilized with 0.01% mercuric chloride. A 2 mm diameter cork-borer was used to make holes into the healthy fruits. Two-millimeter disc of the pure culture of each isolate was cut and placed in the hole. Tissues were replaced and wounds were sealed with sterile vesper prepared from wax and Vaseline. The controls were set up in the same manner except that sterile agar was used instead of the isolate. The plates were incubated at room [28].

# Sample Preparation for Determination of Nutrient composition

In the laboratory, the apparently healthy banana fruits were carefully separated into mesocarps (edible pulp) and epicarp (peel). A portion of each of the samples were thinly sliced and dried at 105°C in an oven. After cooling, the dried samples were ground to powder form and kept air tight in previously washed, dried and labeled sample containers. This sample powder was used for crude protein, carbohydrate, crude fiber, ash and lipid extract determinations. The remaining fresh portion was used for vitamins determinations.

**Determination of moisture.** The moisture content of the banana sample was determined using the oven drying method [18].

**Determination of ash**. Ash content was determined in triplicates as described by Forster *et al.* [5].

**Determination of crude fiber.** Crude fiber was determined using the method of Nielsen [18].

**Determination lipids.** Lipid contents were determined using the method of Nielsen [18].

**Determination of crude protein.** Crude protein was determined using the method of Krohn [11].

**Determination of carbohydrates.** The total carbohydrate contents in the samples were determined using the method of difference [19]. Thus, percentage available carbohydrate was obtained as: % carbohydrate = 100 - (% moisture + % ash + % crude protein + % lipid extract + % crude fiber.

**Determination of vitamins (B<sub>2</sub>, B<sub>9</sub> and C).** Vitamins (B<sub>2</sub>, B<sub>9</sub> and C) were analyzed in the apparently healthy and infected banana fruits using UV-Visible spectro-photometry using the method of Monakhova [15].

Folic acid ( $B_9$ ). Absorbance for folic acid was read at 273.00 nm using UV-Visible spectrophotometer (Shimadzy UV-2550). Folic acid standards were prepared and calibration curve of absorbance against concentration plotted. Concentrations of the samples were read from the calibration curve.

**Riboflavin (B<sub>2</sub>).** Absorbance for riboflavin was read at 261.00 nm. Riboflavin standard solutions were prepared and a calibration curve of absorbance against concentrations plotted. Concentrations of the samples were obtained from the calibration curve.

Ascorbic acid (C). Absorbance for ascorbic acid was read at 478.50 nm. Standard ascorbic solutions were prepared and a calibration curve of absorbance against concentration plotted. Concentrations of the samples were obtained from the calibration curve.

### RESULTS

Colletotrichum musae, Aspergillus niger, Aspergillus flavus and Rhizopus stolonifer are the pathogenic fungi identified on the infected banana fruits. A total of forty-eight fungal isolates were obtained from the banana samples collected from Ahaohu market. Colletotrichum musae was the highest occurring species with 17 (34.4%) followed by Aspergillus niger 13(27.1%). The third occurring colonies was Aspergillus flavus 10(20.8%) while the least occurring isolate was Rhizopus stolonifer 8(16.8%) (Table 1).

### Pathogenecity Test

The pathogens were present in all cases of the disease. The same pathogens were isolated from the diseased host and grown in pure culture. When inoculated into a healthy sample of banana fruits, the pathogens from the pure culture caused the same disease. The same pathogen was re-isolated from the new host and shown to be the same as the originally isolated pathogens (Table 2).

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Table 1. Fungi isolated from the Banana fruits obtained at Ahaohu market in Abakaliki

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Fungi isolated	Total number of fungi isolated	%
C. musae	17	35.4
A. niger	13	27.1
A. flavus	10	20.8
R. stolonifer	8	16.7
Total	48	100

Table 2. Pathogenicity test after inoculation on fresh healthy banana fruits and incubation for 4 days

Fungal pathogen	C. musae	A. niger	A. flavus	R. stolonifer
Test	+++	++	++	+

Key: +++ is the most active pathogen of banana fruits

++ is active pathogen of banana fruits

+ is weak pathogen of banana fruits

Table 3. The result showing the proximate composition (%) of apparently healthy and infected banana fruits

Nutrient identity	Infected banana	Apparently healthy
(%)	fruit	banana fruit
Crude protein	2.63±0.02	$2.66 \pm 0.02$
Carbohydrate	19.54±0.06	20.92±0.01
Lipid extract	$0.20\pm0.02$	0.21±0.07
Ash	0.91±0.02	$0.97{\pm}0.04$
Crude fibre	$0.88{\pm}0.02$	$0.90{\pm}0.05$
Moisture	75.85±0.01	$74.34{\pm}0.05$

Table 4. The result showing the concentration of vitamins (mg/100mg) in apparently healthy and infected banana fruits

s/no	Vitamins	Apparently healthy banana fruit	Infected banana fruit
1	Vitamins B <sub>2</sub>	1.89	1.35
2	Vitamins B9	29.41	23,51
3	Vitamin C	22.44	19.45

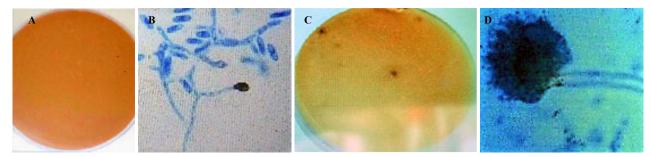


Figure 1. A - is the old culture of Colletotrichum musae; B - is the photomicrograph of the C. musae (×400); C - is old culture of Aspergillus niger while; D - is the photomicrograph of A. niger (×400).

# Proximate and vitamin compositions of the banana fruits

The results on proximate and vitamin compositions of the banana fruits are presented in Tables 3 and 4 below, showing the percentage of crude protein; carbohydrates; lipid extracts; ash, crude fiber, moisture with vitamins.

### DISCUSSION

In this study out of the 48 fungal isolates obtained during the study, *Colletotrichum musae* was the most frequently occurring fungal isolate of the banana sample with the percentage occurrence of 35.4%. While the least occurring isolate was *Rhizopus stolonifer* with percentage occurrence of 16.7%. The result of the pathogenicity test shows the pathogens as originally isolated pathogen of banana sample (Tables 1 and 2). Yahaya *et al.* [27] reported that fungi are the most common cause of spoilage on fruits and vegetables and several fungi like *Colletotrichum* spp., Cladosporium spp., and Fusarium spp. were known to cause large scale storage loss of fruits and vegetables from harvest to storage. Tables 3 and 4 show the results of the proximate and vitamin analyses of the fungal-infected and apparently healthy banana fruits. From the results, there was increase in the moisture content of the fungal infected banana fruits relative to apparently healthy ones. Crude protein, the carbohydrate, lipid extract, crude fiber and ash contents of the infected banana fruits were low compare to apparently healthy ones. The high percentage of moisture in the infected banana fruits may be as a result of extra-cellular digestion and decomposition of the banana fruit tissue into a mush by C. musae and A. niger as Zakari et al. [28] reported similar case from their study. The increase in the moisture content might also be as a result of deleterious reduction in the crude protein, carbohydrate, lipid extract, crude fiber and ash contents of infected banana fruits. It is clear in Table 1 that lipid extract and crude fibre are extremely low in both infected and healthy banana fruits. In other words,

fungal infected and apparently healthy banana fruits contain very low lipid and fibre. This report of low lipid and fibre content of the banana fruits in this study is in agreement with the report of Oyeyinka and Afolayan [20]. The reduction in the nutritional composition of the fungal infected banana fruits is a backup to Bonner [2] report, that polysaccharides and protein are required by fungi for building the hyphal wall and for respiration to obtain energy. Subash and Saxena [24] reported that reduction in the nutritional composition of fruits brings about increase in their chemical contents like phenol while Zakari et al. [28] reports that consumption of the fungal infected fruits by man could lead to such vitamin deficiency diseases as scurvy, dry skin and dermatitis type effect, since the fungal pathogens have utilized the vitamin content of the fruits for their growth.

It is now specific and clerarer that *C. musae, A. niger A. flavus* and *R. stolonifer* are the two common pathogenic fungi which cause reduction in the nutritional composition of banana fruits. Consumption of the fungal infected fruits by man could lead to deficiency diseases as scurvy, dry skin and dermatitis type effect, since the fungal pathogens have utilized the nutrient contents of the fruits for their growth. The study is partially novel as no report of this kind has been available from Abakaliki metropolis. This study has shown that banana fruits contain high level of Vitamin B<sub>9</sub> (folic acid) with low lipid and fibre; this will help the general public, especially, fruitarians and dietitians to make proper combination of their foods.

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