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Influence of African Cassava Mosaic Disease (ACMD) on the Proximate, Mineral and Anti-Nutrient Composition of the Stem Cuttings of *Manihot esculenta* CRANTZ

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ABSTRACT
The utilization of the stem cuttings of most cassava varieties for agricultural and research purposes are sometimes limited due to disease attack and poor understanding of their nutritional and anti-nutritional status. In this research, test plants were sourced and propagated in a completely randomized design method. Five months post propagation (PP), infected and healthy stem cuttings (0 – 20cm) of *M. esculenta* were obtained and analyzed for elemental, proximate and anti-nutrient contents using standard protocols as described by Association of Official Analytical Chemist. The results of elemental composition revealed that ACMD caused significant reductions ($P < 0.05$) in most elements assayed. The mean values of 13.83 ± 0.31, 11.25 ± 0.20 and 27.30 ± 0.30 mg/100g were obtained for phosphorus, calcium and iron in infected sample against the values of 25.08 ± 0.41, 16.20 ± 1.11 and 31.63 ± 0.10 mg/100g for healthy sample. Besides lipid and ash amounts, other proximates analyzed were significantly ($P < 0.05$) lower in the diseased than healthy sample. The ACMD-infected plant showed significant increases in all the anti-nutrient values studied when compared with the corresponding values obtained for the uninfected plant. ACMD can be controlled by the use of resistant varieties and adherence to best phytosanitational measures during and after propagation.

INTRODUCTION
Cassava (*Manihot esculenta* Crantz) originated in Central and South America (Ohadike, 2007), but is now widely grown in tropical and subtropical regions including those of Africa, Madagascar, India, Indonesia, Malaysia, Thailand and the Philippines. It is a perennial woody shrub of the family Euphorbiaceae. Cassava was introduced into West Africa by the Portuguese in the late 16th century via Sao Tome, Fernando Po and the Congo River, but its early spread was slow (Ohadike, 2007). Although the crop is often regarded primarily as a famine reserve, there has been increasing realization in recent years of its value as a high yielding source of carbohydrates (Nweke, 1997). The reported production of 56 million metric tonnes, grown on 7.5 million hectares, represents 43% of the world total and is a major food item for at least 200 million African people (FAO, 2005). The average yield of cassava in Africa – 7 to 8 tonnes per hectare – is far below the potential of the crop. The most important single reason for this is probably the almost ubiquitous presence of African cassava mosaic disease (ACMD). ACMD is caused by African cassava mosaic virus (ACMV). The virus is transmitted by white flies and also by vegetative propagation (using cuttings from infected plants) and occasionally by mechanical means (Fargette and Thresh, 1995; Timmermans *et al.,* 1994). Pants infected with ACMD are not killed but show pale green or yellow areas on the leaves, which are commonly small and distorted. Tubers are reduced in size and number. Stem diameter and overall size are also reduced. Reports of attack and the effects of ACMD on various aspects of *M. esculenta* abound in literature (Arora *et al.,* 1990, Aiton *et al.,* 1998, Hull, 2002, Ogbe, 2003). However, information on the

influence of ACMD on the proximate, mineral and anti-nutrient composition of the stem cuttings of *M. esculenta* is scanty hence, the need for the present study.

MATERIALS AND METHODS

Sources of Sample Collection

Improved and virus free cassava stem cuttings (*Tropical manihot esculenta* – TME 419) were sourced from Akwa Ibom State Agricultural Development Programme (AKADEP) Centre in Uyo, Nigeria. ACMD infected stem cuttings of the local variety of TME with characteristic symptoms similar to those previously reported by Anon (1993) and Ogbe *et al.* (2003) were obtained from a cultivated farmland in Ibiono Ibom Local Government Area of Akwa Ibom State.

Experimental Design and Propagation of Test Plants

The propagation of TME 419 and infected cassava stem cuttings were performed in July, 2018 in a marked out and cleared loamy soil located at Ikot Udo Village in Ibiono Ibom Local Government Area of Akwa Ibom State, Nigeria. Two stem cuttings were placed flat in a wide shallow holes of depth (4.00 cm) spaced one metre square (Udoh *et al.,* 2005). They were covered with soil and monitored for sprouting and growth development. The experiment was laid out in a complete randomized design with ten rows of infected plants while another ten rows of healthy plants served as control, each with five replicates.

Sample Collection and Treatment

Five months post propagation (PP), infected and healthy stem cuttings (0 – 20 cm) of *M. esculenta* were obtained and washed with tap water. Samples were further cut into tiny pieces, dried and reduced to fine power (20 meshes), mixed to obtain homogenate samples and stored in desiccators, protected from light, until further analysis.

Proximate Analysis

The moisture contents of the stem cuttings of *M. esculenta* were determined by drying 5 g of the samples (in triplicates) in a gallen kamp oven at 105^{O}C until constant weights were obtained (AOAC, 2006). Ash contents were determined by dry ashing in lenton muffle furnace at 600^{O}C until grayish white ash was obtained. Crude protein was determined by multiplying the value obtained from Kjeidahl's nitrogen by a protein factor of 6.25 (AOAC, 2006). Crude lipid was quantified by the method described by AOAC (2006) using the soxhlet apparatus and petroleum ether (B.P. 60^{O}C – 80^{O}C) as a solvent. Crude fibre was determined by acid-bases digestion with 1.25% H_2SO_4 (W/V) and 1.25% NaOH (W/V) solutions. Available carbohydrates were calculated by difference, i.e. total sum of crude protein, crude lipid, crude fibre and ash deducted from 100% dry matter (AOAC, 2006).

Mineral Analysis

The method of AOAC (2006) was employed for the determination of mineral contents. 1.0 g of each pulverized samples was placed in a crucible and ignited in a muffle furnace at 550^{O}C for 6 hours. The resulting ash was dissolved in 10 ml of 10% HNO_3 and heated slowly for 20 minutes. After heating, it was filtered and the filtrate was used for the

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determination of mineral contents. Atomic absorption spectrophotometer (AAS) was used to determine phosphorus, calcium, iron, zinc, magnesium and copper while flame photometer was used for the determination of Na and K in each sample.

Quantitative Anti-nutritional Analysis

Determination of Oxalate

The titrimetric method of Day and Underwood (1986) was used in the determination of oxalate in each of the sample. 150 ml of 15 N H_2SO_4 was added to 5 g of the pulverized sample and the solution was carefully stirred intermittently with a magnetic stirrer for 30 minutes and filtered using Whatman No. 1 filter paper, after which 25 ml of the filtrate was collected and titrated against 0.05 M standardized $KMno_4$ solution until a faint pink colour appeared that persisted for 30 seconds.

Determination of Phytate

The phytate content was determined using a modified indirect colorimetric method of Wheeler and Ferre (1971). The method depends on an iron to phosphorus ratio of 4:6 and is based on the ability of standard ferric chloride to precipitate phytate in dilute HCl extract of the sample. 5 g of the sample was extracted with 20 ml of 3% trichloroacetic acid and filtered. 5 ml of the filtrate was used for the analysis; the phytate was precipitated as ferric phytate and converted to ferric hydroxide and soluble sodium phytate by adding 5 ml of 1 M NaOH. The precipitate was dissolved with hot 3.2 M HNO_3 and the absorbance were read immediately at 480 nm. Preparation of standard curve for phytic acid was done as follows: standard curve of different Fe $(NO_3)_3$ concentrations was plotted against the corresponding absorbance of spectrophotometer to calculate the ferric iron concentration. The phytate phosphorus was calculated from the concentration of ferric iron assuming 4:6 iron phosphorus molar ratio.

Determination of Cyanide

Cyanide content was determined by alkaline picrate method according to (AOAC, 2006). 5 g of powdered sample was dissolved in 50 ml of distilled water in a corked conical flask and the extraction was allowed to stand over-night, filtered. 1 ml of sample filtered was mixed with 4 ml alkaline picrate in a corked test tube and incubated in a water bath for 5 mins. After colour development (reddish brown colour), the absorbance were read at 490 nm, the absorbance of the blank containing 1 ml distilled water and 4 ml alkaline picrate solution was recorded. The cyanide content was extrapolated from cyanide standard curve prepared from different concentrations of KCN solution containing 5 – 50 Ng cyanicde in a 500 ml conical flask followed by addition of 25 ml of 1 NHCl.

Determination of Tannin

A 0.5 g portion of the sample was measured into a 50 ml beaker. 20 ml of 50% methanol was added and covered with parafilm and placed in a water bath at 77 – 80^{O}C for 1 hour. It was shaken thoroughly to ensure a uniform mixture. The extract was quantitatively filtered using a double layered Whatman No. 1 filter paper into a 100 ml volumetric flask, 20 ml of water was added, 2.5 ml Folin-Denis reagent and 10 ml of 17% Na_2CO_3 were added and

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mixed properly. The mixture was made up to the marked level with distilled water mixed well and left undisturbed for 20 minutes for the development of a bluish-green colour. The absorbance of the tannic acid standard solutions as well as the samples were read after colour development on a UV-Vis spectrophotometer model 752 at a wavelength of 760 nm (AOAC, 2006).

Statistical Analysis

All determinations were carried out in triplicates. The results generated from the analysis were subjected to statistical analysis using the statistical package for social science (SPSS) version 17. Description statistics was used to interpret the results obtained.

RESULTS

The influence of the ACMD in stem cuttings of *M. esculenta* are shown in Table 1. The results revealed that most elements analyzed were significantly ($P<0.05$) reduced by the disease. The mean values of 13.83 ± 0.31, 11.25 ± 0.20 and 27.30 ± 0.30 mg/100g were obtained for phosphorus, calcium and iron in infected sample against the values of 25.08 ± 0.41, 16.20 ± 1.11 and 31.62 ± 0.10 mg/100g recorded for the healthy sample. Besides lipid and ash composition, other proximates analyzed were significantly ($P<0.05$) lower in the diseased than healthy plant (Fig. 1a and b). The ACMD-infected plant showed significant increases in all the anti-nutrient values studied when compared with the corresponding values obtained for the uninfected plant (Table 2).

Table 1: Elemental composition in stem cuttings of *M. esculenta* infected by ACMD

Element	Infected (mg/100g)	Healthy (mg/100g)
Phosphorus (P)	13.83±0.31*	25.08±0.41
Potassium (K)	8.02±0.10	9.30±0.35
Sodium (Na)	10.35±0.10	11.33±0.02
Calcium (Ca)	11.25±0.20*	16.20±1.11
Iron (Fe)	27.30±0.30*	31.63±0.10
Zinc (Zn)	0.08±0.21*	0.40±1.02
Magnesium (Mg)	2.86±0.20*	1.59±0.30
Copper (Cu)	0.30±0.01*	0.70±0.02

Values are mean ± SD, n = 3 replicates $P< 0.05$, *Significant

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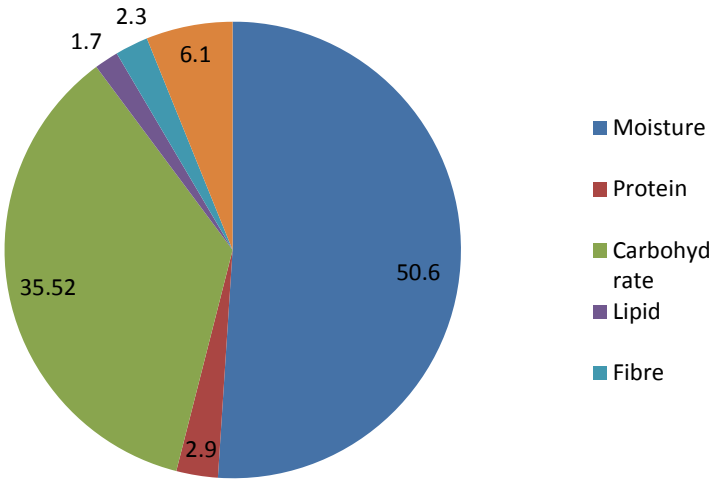

Figure 1a: *Effects of ACMD on proximate composition in infected M. esculenta stem cuttings*

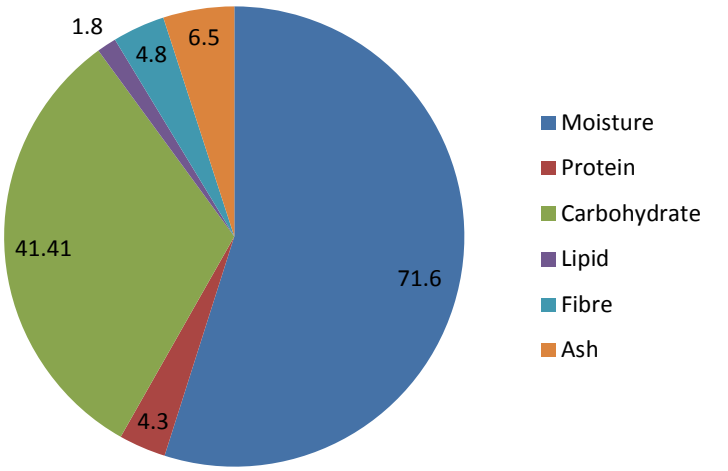

Figure 1b: *Proximate composition in healthy stem cuttings of /. .. esculenta*

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Table 2: Anti-nutrient contents in stem cuttings of *M. esculenta* infected by ACMD

Parameters	Infected (mg/100g)	Healthy (mg/100g)
Oxalate	12.35±0.29*	9.40±1.01
Phytate	11.40±1.11*	6.83±1.20
Hydrogen cyanide (HCN)	140.65±0.14*	120.20±0.02
Tannin	20.20±1.41*	17.41±0.34

Values are mean ± SD, n = 3 replicates P< 0.05, *Significant

DISCUSSION

Cassava *(Manihot esculenta)* is an important part of diet which forms the growing trade between developed and developing countries. Despite its enormous potentials, the production of specific varieties seems limited due to certain factors. This research investigated the influence of African cassava mosaic disease (ACMD) on the mineral proximate and elemental composition in stem cuttings of *M. esculenta*. From the present study, ACMD caused significant reductions in the composition of most elements analyzed. This is similar to the findings of other researchers involving plants mosaic attack (Stylianidis *et al.,* 2005; Yardimci *et al.,* 2006; Muguit *et al.,* 2007). Significant reductions in phosphorus, calcium, iron, magnesium and copper in the infected plant could be due to the possible adverse effects of the disease and alterations in plant metabolism.

Phosphorus is essential part of photosynthesis, helps in the transformation of solar energy, proper plant maturation withstanding stress, effect rapid growth, encourages blooming and root growth. Calcium is always present in green plants. It occurs in the cell-wall, particularly in the middle lamella, as calcium pectate. It helps maintain the semi-permeability of the protoplasm (Dutta, 2009). In general, its absence stunts growth in plants. Magnesium in plants helps in the synthesis of phosphorus containing lipid substances (phospholipids), which are important constituents of protoplasm (Dutta, 2009). In plants nutrition, potassium is known to act as a catalytic agent in the synthesis of carbohydrates and proteins. It helps the plant grow and enables it to produce healthy flowers, seeds and fruits. Sodium, zinc and copper are constituents of certain enzymes (Mehrotra and Aggarwal, 2006). Iron is essential for oxidation reduction in respiration.

This study has shown significant (P<0.05) reductions in quantities of proximates in infected than healthy sample. Increases or decreases in proximate contents of the diseased plants have been reported when compared with those of healthy plants (Leal and Lastra, 1984; Mofunanya *et al.,* 2008; Sinha and Srivastava, 2010). Disproportionate accumulations of proximate in infected plant samples are common feature of disease attack probably due to biotic stress. In plant nutrition, lipids are used for energy and often stored as energy reserves. Moisture or water is an excellent solvent. Many nutrients which enter plants are dissolved in it. Fibre constitutes the structural components of the stem and leaf. Glucose, the sugar produced by plants, is an important carbohydrate. Glucose and most other carbohydrates are energy source for both plants and animals. Research proofs that ash signifies the level of mineral elements present in a plant material, since it has a direct relationship with elemental mineral content (Edem *et al.,* 1984). In the present

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investigation, ACMD caused significant increases ($P<0.05$) in all the anti-nutrient contents of infected plant. One major factor limiting the wider food utilization of many tropical plants is the ubiquitous occurrence in them of a diverse range of natural compounds capable of precipitating deleterious effects in man and animals often referred to as anti-nutritional factors (Shanthakuman *et al.,* 2008). Phytates (also known as Inositol hexakisphosphate Insp6) is the salt form of phytic acid, are found in plants, animals and soil. In addition, phytate has been suggested to serve as a store of cations of high energy phosphoryl groups, and by chelating free iron, as a potent natural anti-oxidant (Mueller, 2001). The oxalate obtained in this research is a salt formed from oxalic acid. It has been found to be widely distributed in plants. Strong bonds are formed between oxalic acid, and various other minerals, such as calcium, magnesium, sodium and potassium (Noonan and Savage, 1999). As anti-nutrients, tannins are known to be present in food products and to inhibit the activities of trypsin, chemotrypsin, amylase and lipase, decrease the protein quality of foods and interfere with dietary iron absorption (Mueller, 2001). A number of plant species produce hydrogen cyanide (HCN) from cyanogenic glycosides of a sugar, often glucose, which is combined with a cyanide containing a glycone. Their general function in plants is dependent on activation by b-glucosidases to release toxic volatile HCN as well as a ketones or aldehydes to fend off herbivore and pathogen attack (Fereidoon, 2014).

CONCLUSION AND RECOMMENDATION

ACMD has caused significant alterations in the composition of most elements, proximate and anti-nutrients content of infected *M. esculenta* when compared with the healthy plant. Based on the findings of this research, the following recommendations are pertinent:

- Use of resistant varieties should be promoted in all cassava growing regions.
- Strict adherence to phytosanitational measures at all stages of pre and post propagation of *M. esculenta.*
- Effort by government to make available to farmers at low cost, improved stem cuttings of *M. esculenta* is recommended.

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