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Growth and Biocentration Studies of Phytochemical and Leaf Chlorophyll Composition of *Manihot esculenta* Crant Infected with African Cassava Mosaic Disease (ACMD)

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ABSTRACT

Mostly grown as a food source in Africa, cassava is the third largest source of carbohydrates in the world and it is considered as a staple food in most African countries. Despite its enormous potentials, African cassava mosaic disease (ACMD) has been causing yield losses in this plant mostly for particular varieties under specific conditions. On the basis of this, it became necessary to probe more into the effects of ACMD on growth and biocentrations of phytochemical and leaf chlorophyll pigments of *M. esculenta.* Test plants were sourced and propagated in a completely randomized design method. Three months post cultivation the diseased and healthy plants were harvested and studied for growth performance using graphical method, weighing and linear measurements. The phytochemicals of both infected and uninfected plants were determined using standard methods as proposed by Association of Official Analytical Chemist whereas the chlorophyll pigments were evaluated by spectrophotometer. The results revealed that ACMD caused significant reductions ($P<0.05$) in all the growth parameters of *M. esculenta* when compared with healthy sample. The mean shoot height of 12.71 ± 0.31 cm was obtained in the diseased sample whereas the healthy sample had 28.0 ± 2.11 cm respectively. There were significant ($P<0.05$) increases and decreases in the amounts of phytochemicals following ACMD infection of *M. esculenta.* All the chlorophyll pigments measured were significantly reduced in ACMD infected plants. There is need to control the spread of ACMD through the use of resistant varieties and adoption of effective agronomic practice in all cassava growing regions.

INTRODUCTION

Mostly grown as a food source in Africa, cassava (*Manihot esculenta* Crantz) is a perennial woody shrub belonging to the family Euphorbiaceae. It was introduced into Central Africa from South America in the 16th century by the early Portuguese exporters (Ohadike, 2007). Cassava is the third largest source of carbohydrates in the world (Fargette *et al.,* 1994). It is a staple food in most African countries and in recent times, cassava production has turned from subsistence to commercial production (Patil and Fauquet, 2009). Many Nigerians derives much of their food and employment from cassava production, processing, marketing and cassava based agro-industrial scheme (Nweke, 1997). The socio-economic benefits of cassava are threatened by plant diseases among which African cassava mosaic disease (ACMD) is one. ACMD is caused by African cassava mosaic virus (ACMV). This virus is transmitted by whiteflies and also by vegetative propagation (using cuttings from infected plants) and occasionally by mechanical means (Fargette and Thresh, 1994; Timmermans *et al.,* 1994).

The severity of the symptoms depends on the cassava variety and these include chlorotic mosaic of the leaves, leaf

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distortion, malformation, and stunted growth. Yield losses attributable to ACMD are estimated at between 28% - 40% (Thresh *et al.,* 1998). Growth distortions, alteration in biocentrations of phytochemical and chlorophyll in plants as a result of attack by viruses have been reported by several workers (El-Dougdough *et al.,* 2007; Muquit *et al.,* 2007; Daurte *et al.,* 2008; Arora *et al.,* 2009 and Ehinmore and Kareem, 2010). There is scanty information on the growth and biocentrations study of phytochemical and leaf chlorophyll of *M. esculenta* infected with African cassava mosaic disease (ACMD). Hence, this necessitated the research.

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.

Effect of the ACMD on Growth Performance

Shoot Height and Number of Leaves: Sixty days post-propagation, the effects of the disease on shoot height was determined by measuring shoot height (cm) from the base to the tip of the plants. Measurements were taken for three times in the replicated plants. The leaves of the diseased and healthy plants were counted visually from each plant. Average of triplicate determinations were considered and recorded in centimetres.

Leaf Area, Length and Width: Leaves of the same age and position on the diseased and healthy plants were harvested in clean polyethylene bags and brought to Biology Laboratory of the Department of Science Technology, Akwa Ibom State Polytechnic, Ikot Osurua. The area was traced on the graph and total area calculated based on the number of squares within the traced region (Ting, 1982). For lengths and widths, the leaves were placed on a clean specimen board before measurements were made and recorded.

Fresh and Dry Weights of Leaves: Leaf fresh weight was taken for each sample by measuring using Blauscal Weighing Balance (DHG 9053A, Ocean Med. England). Leaf dry weight was determined by drying leaf samples at

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temperature of 70°C for 24 hours. Samples were dried and weighed three times.

Fresh and Dry Weights of Shoots: The harvested plants were placed in a bucket of water and the soil particles gently washed off. The shoots were cut off from the roots using a clean machete and then fresh weights determined and recorded. The samples were later oven-dried at 70°C for 24 hours. They were then weighed three times for both the diseased and healthy plants (Miyashi *et al.,* 1996).

Quantitative Phytochemical Analyses

Alkaloid Determination: The presence of alkaloid in the diseased and healthy samples of *Manihot esculenta* were determined by weighing 5 g of the samples into a 250 cm^3 beakers and 200 cm^3 of 29% acetic acid in ethanol was added and covered to stand for 6 hours. These were filtered and the extracts were concentrated using a water bath to one quarter of the original volume. The alkaloid was precipated out using concentrated ammonium hydroxide which was added drop by drop until precipitations were complete. The solutions were allowed to settle and the precipitations were collected by filtration using whatman filter paper, the precipitates were dried and weighed (Obadoni and Ochuko, 2001).

Saponin Determination: 20 g of the sample was each weighed into a 250 cm^3 beakers and 200 cm^3 of 20% ethanol was added and stirred using a glass rod. The mixtures were heated over water bath for 4 hours with continuous stirring while the temperatures were maintained at 55°C. The mixtures were extracted and the residues were retracted with 200 cm^3 of 20% ethanol. The combined extracts were reduced to 40 cm^3 over water bath at 90°C. The concentrated extracts were transferred into a 250 cm^3 separation funnels and 20 cm^3 of diethyl ether was added and shaken vigorously. The aqueous layers were recovered while the ether layers were discarded. This process was repeated thrice. 60 cm^3 of n-butanol was added. The mixtures were washed twice with a 10 cm^3 of 5% sodium chloride. The remaining solutions were heated over water bath and the residues dried to constant weight. The saponin contents were calculated in mg/100 g (Obadoni and Ochuko, 2001).

Flavonoid Determination: 10 g of the plant samples were extracted repeatedly with 100 cm^3 of aqueous methanol at room temperature. The solutions obtained were filtered with whatman filter paper No. 45. The filtrates were later transferred into a crucible and evaporated to dryness over a water bath and weighed (Boham and Kocipai, 1994).

Tannin Determination: 0.5 g of the samples was each weighed into 250 cm^3 beakers and 50 cm^3 of distilled water was added and stirred vigorously with a glass rod for one hour. The solutions were filtered into a 50 cm^3 volumetric flask and made up to mark. 5 cm^3 of the filtrates were pipetted into test tubes and mixed with 3 cm^3 of 0.1 M $FeCl_3$ in 0.1 N HCl and 0.008 potassium Ferro cyanide. The absorbance were measured with the Jenway digital spectrophotometer model 6303 at 120 nm wave length. The absorbance were compared with those of standard made from tannic acid (Van-Burden and Robinson, 1981).

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Phenol Determination: 2 g of the sample each was defatted with 100 cm^3 of diethyl ether using a soxhlet apparatus for two hours. The defatted sample was boiled within 50 cm^3 of ether for 15 minutes, then 5 cm^3 of the extract was pipetted into a 50 cm^3 flask and 10 cm^3 of distilled water was added 2 cm^3 of ammonium hydroxide and 5 cm^3 of amyl alcohol was added. The samples were made up to the mark and left for colour development. The absorbance of the solution was measured using Jenway digital spectrophotometer model 6303 at 505 nm wavelength (Obandni, 2001 and Harbone, 1973).

Quantitative Determination of Chlorophyll

The effects of African cassava mosaic disease (ACMD) on chlorophyll contents of *M. esculenta* were determined at the intervals of 7, 14, 21, 28 and 35 days after one month of propagation. A 2.0 g of each fresh sample was homogenized separately using pestle and mortar in 10 ml of 80% acetone. The homogenates were poured into test tubes and centrifuged at 4000 rpm for 3 minutes. The filtrates were decanted and used for chlorophyll determinations. The blank was prepared and set the instrument to zero with 3 ml of acetone. The absorbance readings were taken spectrophotometrically (Strickland and Parsons, 1972).

DATA ANALYSIS

Data were expressed as mean ± S.EM of three replicates and subjected to analysis using statistical package for social science (SPSS) version 17.0 as described by Ubom (2004).

Table 1: Growth Performance of *Manihot esculenta*

Parameters	Diseased Plant	Healthy Plant
Shoot height (cm)	12.71 ± 0.31*	28.01 ± 2.11
Leaf number	15.00 ± 0.01*	19.11 ± 0.20
Leaf length (cm)	6.20 ± 1.04*	11.40 ± 0.13
Fresh shoot weight (g)	10.40 ± 1.01*	23.00 ± 1.11
Dry shoot weight (g)	3.90 ± 2.44*	9.91 ± 1.31
Fresh leaf weight (g)	5.30 ± 1.22*	11.40 ± 2.00
Dry leaf weight (g)	2.00 ± 0.11*	5.30 ± 0.30
Leaf area (cm^2)	6.20 ± 1.00*	8.11 ± 3.01

Values are means ± S.EM, n = 3 replicates, $P<0.05$ *Significant.

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Table 2: Quantitative Phytochemical Contents of *Manihot esculenta* Leaf

Chemical Constituents (mg/100g)	Diseased Sample	Healthy Sample
Alkaloids	2.41 ± 0.01*	4.58 ± 0.01
Saponnins	3.50 ± 0.03*	2.20 ± 0.02
Flavonoids	10.01 ± 0.03*	13.41 ± 0.06
Tannins	3.10 ± 0.03*	3.62 ± 0.04
Phenols	18.20 ± 0.07*	14.10 ± 0.01

Values are means ± S.EM, n = 3 replicates, P<0.05 *Significant.

Table 3: Effects of African Cassava Mosaic Disease (ACMD) on Chlorophyll Contents of *M. esculenta*

Days Post Propagation (DPP)	Composition	Diseased Sample (mg/FW)	Healthy Sample (mg/FW)
7	Chlorophyll a	101.20 ± 1.30*	118.30 ± 1.10
	Chlorophyll b	115.00 ± 1.00*	110.0 ± 1.33
	Total Chlorophyll (a + b)	**216.2**	**228.3**
14	Chlorophyll a	92.03 ± 0.11*	120.14 ± 0.00
	Chlorophyll b	100.10 ± 3.20*	116.00 ± 1.11
	Total Chlorophyll (a + b)	**192.13**	**236.14**
21	Chlorophyll a	84.11 ± 0.01*	124.00 ± 1.00
	Chlorophyll b	91.00 ± 1.22*	119.20 ± 1.06
	Total Chlorophyll (a + b)	**175.11**	**243.2**
28	Chlorophyll a	79.20 ± 2.11*	129.47 ± 3.11
	Chlorophyll b	87.00 ± 2.20*	126.20 ± 0.33
	Total Chlorophyll (a + b)	**166.2**	**255.67**
35	Chlorophyll a	71.33 ± 1.12*	134.31 ± 2.03
	Chlorophyll b	80.11 ± 0.00*	129.00 ± 1.34
	Total Chlorophyll (a + b)	**151.44**	**263.31**

Values are means ± S.EM, n = 3 replicates, P<0.05 *Significant.

RESULTS

The growth performance of *M. esculenta* are summarized in Table 1. The results revealed that ACMD caused significant reductions (P<0.05) in all the growth parameters of *M. esculenta* when compared with the healthy sample. The mean shoot height of 12.71 ± 0.31 cm was obtained in the diseased plant whereas the healthy sample had the corresponding mean value of 28.01 ± 2.11 cm. For quantitative phytochemical contents, alkaloids, flavonoids, and tannins in the infected *M. esculenta* were significantly decreased (P<0.05) whereas saponins and phenols shared significant increases in the diseased sample when compared to the healthy one (Table 2). There was a general reduction in the mean values of chlorophyll composition found in the diseased plant when compared to the corresponding high values obtained for healthy plant (Table 3). At 7 DPP chlorophylls a and b in the diseased plant were 101.20 ± 1.30 and 115.00 ± 1.00 (mg/FW) whereas the uninfected sample recorded 118.30 ± 0.10 and 110.0 ± 1.33 (mg/FW) respectively. At the end of the experiment (35 DPP) the mean values of chlorophyll contents in the infected *M. esculenta* showed greater reductions than those of the healthy sample.

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DISCUSSION

This research presents "Growth and biocentrations study of phytochemical and leaf chlorophyll of *M. esculenta* infected with African cassava mosaic disease (ACMD)". From this study, the disease caused significant reductions in all the growth parameters of the infected plant. The observed effects of ACMD on *M. esculenta* in this work correspond with the work of Pawer *et al.* (1990) who reported reductions in shoot height, leaf weights as well as the leaf number of sorghum infected with sorghum ringspot virus (SRSV). Similarly, Ehinmore and Kareem (2010) reported reduced growth rates in three *Amaranthus cultivars* as a result of disease attack. Plant diseases are always known to cause some deviations from normal growth of plant, reduction in plant height, size of leaves, flowers and roots, and shortening of petioles and internodes (El-Dougdoug *et al.,* 2007). Growth in plants is a complex phenomenon linked with numerous morphological and physiological processes. Phytochemical alterations observed in the present study are in line with those reported by previous workers (Wood, 1990; Fallah *et al.,* 2005; Edeoga and Erita, 2006 and Duarte *et al.,* 2008). Phytochemical like alkaloids are beneficial chemicals to plants serving as repellant to predators and parasites. This probably endows these group of agents its antimicrobial activity. Several alkaloid containing medicinal plants are reported to have been used by the early man as pain relievers, as recreational stimulants or in religious ceremonies to enter a psychological state to achieve communication with ancestors or God (Gurib-Fakin, 2006).

Saponins in medicinal plants are responsible foremost biological effects related to cell growth and division in humans (Yadav and Agarwala, 2011). They have inhibitory effect on inflammation (Okwu and Okwu, 2004). Flavonoids are distributed group of polycyclic compounds characterized by a common Benzo pyrome ring structure that has been reported to act as antioxidants in many biological systems (Del Rio *et al.,* 1997; Okwu and Okwu, 2004). The increased in the phenolic contents of infected *M. esculenta* observed in this research suggest that their synthesis is stimulated by stress. Many phenolics are known to exhibit antioxidant properties; they are free radical's scavengers. Tannins have astringent properties, hastening the healing of wounds and inflamed mucous membrane (Okwu and Okwu, 2004). In the present study, altered and the general reductions of chlorophyll pigments in infected *M. esculenta* have been observed. These findings are in concurrent with a general decrease of chlorophyll composition in disease infected plants which have been reported (Szigeti *et al.,* 2002; Sameh, 2005; Arora *et al.,* 2009 and Sinha and Srivasta, 2010). Chlorophyll a and b are the two most abundant chlorophylls. They are light harvesting arrays, absorbing and transferring excitation energy to photochemical reaction centres of photosystems I and II where their light energy is converted into chemical free energy.

CONCLUSION

In conclusion, this study has shown that African cassava mosaic disease (ACMD) caused a general growth

distortions in infected *M. esculenta* as well as alteration and reduction in basic phytochemicals and leaf chlorophyll concentrations.

RECOMMENDATIONS

To prevent the spread of ACMD in cassava growing regions, the following recommendations are pertinent:

1. Phytosanitation of all stages of plant propagation is paramount.
2. The use of resistant varieties should be encouraged.
3. Government should open more quarantine offices both in urban and rural areas for certification of plant materials.

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