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Evaluation of the Effect of *African cassava mosaic virus* (ACMV) Genus *Begomovirus* Infection on the Nutritional Components of Cassava (*Manihot esculenta.* Crantz)

Oben Tom Tabi[1*], Egbe Enow Andrew[1, 2] and Atiri Gabriel Ifo[3]

[1]*Department of Agronomic and Applied Molecular Sciences, Faculty of Agriculture and Veterinary Medicine, University of Buea. PMB 63, Buea, Cameroon*
[2]*Department of Plant Science, Faculty of Science, University of Buea. PMB 63, Buea, Cameroon*
[3]*Department of Crop Protection and Environmental Biology, University of Ibadan, Ibadan, Nigeria*

**Corresponding author*

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A B S T R A C T

Cassava is an important staple grown worldwide for its edible leaves and roots. *African cassava mosaic virus* (ACMV) is the most important disease infecting this crop that reduces yield and quality. This study was conducted to determine the effect of ACMV infection on nutritional components of some cassava cultivars. The experiment was carried out at the University of Ibadan, Teaching and Research Farms. It was a randomized complete block design with four replicates using resistant, moderately resistant and susceptible cultivars. Proximate analysis was done to evaluate these nutritional components. Disease incidence and severity were highest for the susceptible [Index of Symptom Severity (ISS) =3.57 ± 0.01] cultivar and lowest for the resistant (1.36 ± 0.05) nine months after planting. Cyanide content was 37.1% for the resistant plants and 78.3% for the susceptible. Fibre and fat contents decrease significantly for infected plants compared to the uninfected irrespective of the cultivar. A decrease of 2.68% in protein content and an increase in biomass of 21.43% was noted for infected resistant plant compared to the uninfected. Index of Symptom Severity (ISS) and protein/sugar contents of cassava tubers were significantly positively correlated (P=0.05) on dry weight basis. There was significant negative correlation between starch/sugar and dry matter/moisture content of cassava tubers (r=0.9 and r=-0.8) respectively. On fresh weight basis, there was a highly significant negative correlation between ISS and moisture content (r=-0.6). Starch content was significantly positively correlated at *P*=0.05. Cyanide and dry matter contents had significant positive correlation (r=0.9, *P*=0.05). ACMV has an adverse effect on the nutritional components of cassava and therefore virus resistant cultivars should be used for planting.

Introduction

Cassava (*Manihot esculenta* Crantz) is a Euphobiaceae commonly called manioc or yucca and grown mainly in the tropical and sub-tropical regions of the world (Wooding *et al.,* 2022). It is a staple in many continents including Central and South America, Asia and Africa and globally, this crop is ranked among the top 10th in production and 6th most important food crop worldwide after rice, maize, wheat, potato and barley (Otekunrin and Sawieka, 2019). Cameroon produces 4,858,329 metric tons of cassava and ranked 13th in the world (Roger *et al.,* 2014; FAOSTAT, 2020).

The root provides carbohydrate and energy to about two billion people worldwide and 700 million for those in tropical and sub-tropical regions (Vincenza *et al.,* 2016). The uses of cassava are enormous: it is processed into a powder-like substance popularly called garri or tapioca, and its flour is used for baking bread and alcoholic beverages. It can be eaten raw or boiled especially those varieties with low cyanide content, it can be processed to local products like water fufu", "akpu", "nkum kum", "meyondoh" and in the production of starch. The leaves are rich in proteins, vitamins and mineral elements are widely consumed as vegetable in sauces (Mouafor *et al.,* 2016). Cassava root contains hydrogen cyanide (Linamarin and lautoustralin) which are toxic especially when consumed in large quantities.

In humans it causes goitre, intestinal neuropathy and endemic ulcer. With production being up to 90 tons/ha, more farmers are getting into its production even though there are several limitations to optimal yield and quality where pests/diseases are of utmost importance and African cassava mosaic disease is one of the most important production constraint.

This virus disease is present in all cassava growing areas in Africa and affects half of all plants and responsible for continent wide losses in excess of $ 1 billion annually making it globally the most important plant virus disease (IITA, 2007). So far, much emphasis has been geared towards increasing the yield of cassava but little or no work has been done to evaluate the effect of ACMVD on the nutritional composition of cassava root. This study therefore, was aimed at evaluating the effect of African cassava mosaic virus disease on the nutritional components of the cassava root.

It has as objectives: to determine the effect of ACMVD on the nutritional composition of uninfected and infected cassava on a dry weight basis; determine the effect of ACMVD on the nutritional composition of cassava on a fresh weight basis and to determine correlation between the ACMVD infection and the nutritional composition of cassava. This study will be far reaching as it will determine the cassava varieties to be recommended for human and livestock consumption.

Materials and Methods

Field layout and experimental design

The experiment was carried out at the University of Ibadan Teaching and Research Farm in Nigeria. It was a randomized complete block design in four replicates. Three cassava cultivars were used: virus-resistant cultivar TMS 30572, moderately resistant cultivar TMS 4(2) 1425 and susceptible Isu-nikankiyan, a local cultivar.

Nine hundred cassava cuttings, i.e. 300 cuttings for each cassava cultivar, with lengths between 25-30 cm and having 6-7 nodes each, were obtained from the International Institute of Tropical Agriculture (ITTA), Ibadan, Nigeria,

The experimental field with a dimension of 30m x 30m was ploughed and harrowed. Cassava cuttings of about one-year old with 6-7 nodes were planted slantly at a spacing of 1m x 1m, depth of l6 -20 cm and at an angle of about 60°. Each of the four replicated blocks had a dimension of 30m x 6m and consisted of three equal plots of 10 m x 6 m. Each plot consisted of 10 rows of cassava plant stands with 7 stands per row. This amounted to 70 cassava

plant stands per plot and 210 stands per replicate. Each plot was separated from each other by an alley with a distance of 1m and the replicates were separated from one another by a distance of 2 m. A total of 840 plant stands were planted in the four replicates.

At planting, no pre-emergence herbicide was used. Weeding was done manually, every month from date of planting until cassava tuber samples were collected from the field ten months later. No fertilizer was used.

Data collection

African cassava mosaic disease (ACMD) incidence and severity for each plot was scored fortnightly beginning from two weeks after planting for six months. The classes of symptom severity were as follows according to IITA (1990a):

1= No symptom

2= Mild chlorotic pattern on entire leaflet or mild distortion of the base of the leaflets, the rest of the leaflets appearing green and healthy

3= Strong mosaic pattern on entire leaf, narrowing and distortion of lower one-third of leaflets

4= Severe mosaic, distortion of two-third of leaflets and general reduction of leaf size

5= Severe mosaic and distortion of four-fifth or more leaflets, twisted and mishappened leaves

The incidence and index of symptom severity (lSS) for each plant was calculated according to Alaux and Fauquet (1990):

Incidence = Number of infected plants/ total number of plants X 100.....eq1

ISS = Sum of severity scores for all the leaves of the plant/ Total number of leaves on the plant.....eq 2

Determination of the nutritional composition of cassava tubers

Sterilization technique

Glasswares such as beakers, crucibles test tubes and burettes, measuring cylinders, centrifuge tubes and volumetric flasks were thoroughly washed with detergent, rinsed in water and allowed to dry. They were then placed in canisters and sterilized in a hot-air oven at 160°C for three hours.

Sample collection and preparation

The uninfected control

Three symptom-free plants (i.e. those with ISS = 1) were harvested from each of the plots. Their tubers were shredded using a kitchen knife, washed to remove all soil, dried in air and 30 g of cassava tuber was collected from each plant, 10g from either ends of the tuber and 10 g from the middle portion.

This was to allow for any longitudinal and radial gradients of composition within the tuber. Ninety grammes was therefore collected from a single plot of each cultivar.

The single cassava cultivar was bulked with the same cultivar in other blocks making a total of 360 g of cassava tuber per cultivar, out of which 100 g was stored at 20°C. This was used for the determination of free and bound cyanide. Two samples of 50 g each of the shredded cassava tuber was dried at 40°C for four days until a constant weight was obtained.

This was used for the determination of the moisture content. The rest of the shredded material was dried at 40°C and ground to a fine white powder using an electric grinder after which the powder was stored in bottles containing a known amount of residual moisture. These samples were used for proximate analysis of protein, sugar, Starch, fibre and fat contents of cassava tuber. The procedure was repeated for the other two cassava cultivars.

Effect of African cassava mosaic disease on the nutritional components of peeled cassava tubers on a dry weight basis: proximate analysis of the nutritional components of mosaic-free and diseased cassava tuber samples

The 15 samples collected were each analyzed for protein, sugar, fibre, fat, moisture, dry matter and cyanide contents. These nutritional components were analyzed on dry and fresh weight basis.

Proximate analysis of the protein content by the Kjeldahl nitrogen method

The principle

This method measured the crude protein content in food because it gives the amount of all the reduced nitrogen in the food in the form of amines, ammonium compounds, urea, amino acids etc. The procedure as described by PanReac (2018) involves digestion of the material with concentrated sulphuric acid (H_2SO_4) and converting the nitrogen to ammonium hydrogen phosphate. The digestion is accelerated by adding a catalyst (potassium sulphate, mercury and copper salts) to increase the boiling point. The mixture is made alkaline by adding sodium hydroxide solution. The ammonium (NH_4) produced is distilled into boric acid. The exact amount of NH_4 is determined by titration with hydrochloric (HCl) acid. Protein values are obtained by multiplying the total nitrogen by a factor of 6.25.

The procedure

One of the samples was put into a digestion tube and 15ml of concentrated H_2SO_4 added. Five Kjeldahl catalyst tablets were added into the tube and the tube put into a digester pre-set at 41^{o}C. This was digested for 45 minutes when a clear blue solution was formed. Seventy-five milliliters of distilled water was added and the tube placed in a distilling unit where 50 ml of 40% NaOH was added. The digest was distilled into a 25 ml of 4% boric acid for 5 minutes to absorb the nitrogen. The distilate was titrated against 0.47 M HCI until a grey colour was obtained. The % total nitrogen and crude protein are given as follows, as described (Yuen and Pollard, 1953):

$$\% \text{ Total Nitrogen} = \frac{14.01 \times (\text{sample titre} - \text{Blank titre}) \times N}{10 \times \text{sample weight.}} \quad \ldots \text{eq 3}$$

N= Normality of the acid

% Crude protein = % total nitrogen x 6.25

Determination of sugar and starch contents of cassava

The procedure followed was as described by (Qiao Lin, 2016). Fifty milligrams of sample was weighed and wet with 1.0 ml 95% ethanol. Twenty ml distilled water was added and the solution mixed. Ten milligrams of hot 80% ethanol was added and mixed thoroughly and this was later centrifuged at 4000 rpm tor five minutes. The supernatant was decanted carefully into a I00 ml volumetric flask and hot ethanol was added to the residue. This was thoroughly mixed and the residue centrifuged again for five minutes and supernatant decanted into a 100ml volumetric flask.

This extraction was repeated with hot ethanol and distilled water added to the combination extracts up to the 100ml mark. The sugar content was determined by the phenol-sulphuric acid method of Dubois (1956) as follows:

One milliliter of sugar extract was pipetted into a test tube and diluted with an equal volume of distilled water. One milliliter of 5% phenol was added and the residue was thoroughly mixed.

Five milliliter of concentrated sulphuric acid was rapidly added directly to the liquid surface in order to obtain good mixing. The tube was allowed to stand for 10 minutes and shaken to mix thoroughly. This was then placed in a water bath at 25^{o}C, absorbance read at 490 nm.

A standard curve was made using 50 mg of glucose. Ten milligrams of glucose was dissolve in 100 ml distilled water; 0.20, 0.40, 0.60, 0.80 and 1.0 ml of standard glucose solution pipetted into a test tube and the procedure repeated as for the determination of sugar.

The residue for the sugar analysis was used for the determination of starch content. This was transferred into a 100 ml volumetric flask with 18 ml of 70% per chloric acid. The acid was divided into three portions in order to rinse the tube thoroughly.

The tube was further rinsed with 9.0 ml water and allowed to stand for two hours. The extract was made up to 100 ml with distilled water and filtered through sintered glass funnel after which 0.2 ml of extract was pipetted into the test tube and diluted to 20 ml with distilled water. The colour developed as in the determination of sugar by the phenol-sulphuric acid method. The amount of sugar was determined by reference to the standard curve, while taking the dilution factor and weight of sample into consideration. Sugar was expressed directly as amount of glucose. To convert to starch, the value of glucose was multiplied by 0.9 (Dubois, 1956).

Determination of crude fibre of cassava by the trichloroacetic acid (TCA) method

The procedure used was that described by Entwistle *et al.,* (1981). One gram of defatted sample was weighed into the digestion beaker and 100 ml TCA digestion agent added. The heating unit of the digester was switched on and water supply to the reflux condenser was opened. The reflux was brought to boiling for exactly 40 minutes counting from time boiling commenced.

The beaker was removed from the heater and cooled. The mixture was filtered using Whatman No. 4. and the residue washed six times with hot water and once with industrial spirit ethanol. The filtered paper was opened out. The residue was removed with a spatula and transferred into a previously ignited and pre-weighed dish. This was dried overnight in an oven at 105°C and transferred to a desiccator and weighed after cooling. It was then ashed in a muffle furnace at 600°C for 6 hours and allowed to cool and weighed. The % fiber was calculated as follows (White and Zarrow, 1945)

% Fiber= Loss in weight of ashing x 100 ...eq 4

Determination of crude fat content of cassava by the soxhlet method

The Principle

This method, as described by Joslyn (1970) measures crude fat which is extracted by solvents such as petroleum ethanol or the extraction is based on the sparing solubility of lipids in water and their separable solubility in non-polar organic solvents. The solvent evaporates off leaving the fat. The measured fat consists of all the soluble materials present in the sample.

Procedure

A 300 ml extraction flask was washed and dried. It was allowed to cool in a desiccator and weighed. The Soxhlet extractor was filled up with reflux condenser and the water flow was done through the condenser. Three grams of sample was weighed on a filtered paper, folded and transferred into a fat-iron extraction thimble which was lightly plugged with cotton wool. The thimble was then placed in the extraction barrel and petroleum ether added in the flask directly below it. The flask was treated with the reflux sample for five hours. The thimble was removed after extraction from the extraction barrel and dried. The barrel was replaced and the solvent distilled off until the extraction flask was almost empty. The flask containing the fat was detached and dried in the oven at a low temperature. The flask containing fat was weighed and the fat content calculated as follows (Joslyn, 1970):

$$\%\,Fat = \frac{(Weight\ of\ flask + Fat) - (Weight\ of\ empty\ flask) \cdot 100}{Sample\ weight.}$$

.....eq 5

Determination of moisture and dry matter contents of cassava

The procedure used was that described by A.O.A.C. (1990). Ten grams of sample was accurately weighed into a pre-weighed clean weighing and drying dish with easily removable lid. Uncovered dish with open lid was placed in a ventilated oven and maintained at 105°C. After 15 hours, the lid was replaced and transferred into a desiccator at room temperature to cool.

0.1 milligram was weighed quickly and the dish was replaced with sample and the lid opened in the desiccator for 2 hours. The lid was replaced, cooled in the desiccator and weighed. This was repeated until a constant weight loss was obtained. The loss in weight was taken as the moisture content of the sample which was calculated as follows (AACC, 1983):

$$\%\,\text{Moisture content} = \frac{M_1 - M_2}{M_1 - M_0} X100 \quad \text{.....eq 6}$$

Where M_0 = Weight in grammes for dish and lid.

M_1= Weight in grammes of lid and sample before drying

M_2- Weight in grammes of lid and sample ater drying

% Dry matter- 100 - % Moisture content...(AACC, 1985)

Determination of cyanagenic potential (CNP) of cassava

The concept

Cassava contains the cyanogenic glucosides linamarin and lotaustralin present in all parts of the plant. These cyanogenic glucosides are hydrolysed to their corresponding cyanohydrins by the enzyme, linamarase when the tissue cellular structure is damaged, for instance during processing of cassava to food products. At pH above 5, cyanohydrins can spontaneously be converted to hydrogen cyanide which readily evaporates even live plants contain only the glucosides; linamarin and lotaustralin. The sum of the concentrations of cyanogenic glucosides, cyanohydrins and cyanide is referred to as CNP

Procedure

This method used was described by Bokanga (1993). Fifteen grams of sample was weighed into 25 ml of 0.1m orthophosphuric acid.

This was homogenized in a blender for 30 seconds at low speed and twice at high speed for a minute. This homogenate was centrifuged and this supernatant contained the extract.

In the determination of CPN, 0.1M of extract was used and treated exactly with linamarin standard. In this way the total CNP was obtained as follows (AACC, 1982):

$$CNP = \frac{A \times 250 \times 0.01095}{B \times X \times W.} \quad \text{.....eq 7}$$

W= Constant value = 58

X= Standard value = 5

B= Weight of sample

A= Sugar reading

Statistical Analysis

The means 1SS for the three treatments in each replicate were calculated for each sampling date and the means of two successive sampling dates pooled. The data was subjected to an analysis of variance (ANOVA). Five ANOVA was thus carried out for the 10 sampling dates in nine months. The comparison of individual means for each month was done using the Duncan's Multiple Range Test.

The mean percentage of each of the nutritional components of cassava tubers studied for the three cassava cultivars of the uninfected plants were compared to those of the infected plants. The comparative analysis for each was done using the t-test.

A Pearson's correlation analysis for the two parameters index of symptom severity and each nutritional component for both the dry and fresh weight bases, was done.

Results and Discussion

African cassava mosaic virus disease symptoms on cassava leaves

In the field, symptoms of ACMVD were observed on cassava leaves for the three cassava cultivars. The mosaic symptoms were characterized by chlorosis of discrete areas of the leaf lamina (Plate 1: A and B) some of the areas fail to expand fully as a result of distortion of the leaflets.

The leaves were twisted and misshapen. The mosaic pattern in some plants was localized in some few leaves but in others all the leaflets showed a nearly uniform mosaic pattern.

Not all the plants of the three cassava cultivars showed symptoms of ACMVD. A greater proportion of the resistant cassava cultivar IMS 30572 showed no disease symptom (Plate 1) while some plants of the moderately resistant cassava cultivar TMS 4(2) 1425 showed no mosaic symptoms. Only a few plants of the susceptible cassava cultivar Isunikankiyan were symptomless.

Monthly means of index of symptom severity (iss) for the three cassava cultivars

In September, the first month of planting, symptoms of ACMD were found in some of the plants in all the three cassava cultivars irrespective of whether the plants were resistant, moderately resistant or susceptible. The symptom severity was lower for the resistant cultivar TMS 30572 (ISS 1.66 ± 0.39) than for the susceptible cultivar Isunikankiyan (ISS 2.58 ± 0.39) (Table 1).

There was no significant difference (P = 5%) in ISS between the resistant and moderately resistant cultivars but there was a significant difference between these two cultivars and the susceptible cassava cultivar (Table 1).

In the second month after planting (October), there was a general increase in symptoms of ACMD both in the number of plants and leaves compared to the first month of planting. The moderately resistant cassava cultivar had the highest ISS difference of 0.27 between the two months as it increased from 1.12 ± 0.39 to 1.30 ± 0.46 (Table 1)

The susceptible cassava cultivar still distinguished itself from the other two cultivars in its ISS rating of 2.96 ± 0.46 being significantly different with respect to the other cultivars (Table 1). The same trend continued in the third month (November) as the resistant cassava cultivar had an ISS value of 0.05, moderately resistant 0.04 and susceptible 0.23 above the previous. (Table 1)

No significant difference in ISS was noted between TMS 30572 and TMS 4 - (2) 1425 but these two cultivars differed significantly from Isunkankiyan (Table 1).

In the fourth month after planting (December), there was generally an increase in ISS for all the three cassava cultivars. There was no difference in ISS for the resistant cassava cultivar between the months of December and January as the rating remained between 1.36 ± 0.54 and 1.36 ± 0.59. Generally, the difference in ISS for the resistant cassava cultivar from the first month of planting (September) to January was quite small as the rating decreased gradually from 1.16 ± 0.39 to 1.36 ± 0.59 (Table 1).

A higher difference was recorded for the moderately resistant cultivar as the ISS values changed from 1.12 ± 0.39 to 1.45 ± 0.39 for the month of September and January respectively. The highest disparity in ISS between the month of September

and January was 0.99 for the susceptible cultivar as values increased sharply from 2.58 ± 0.39 to 3.57 ± 0.59 (Table 1).

Effect of African cassava mosaic virus disease on the protein content of peeled cassava tubers from uninfected and infected plants

Protein composition on dry weight basis

Generally, there was an increase in the protein content of tubers from infected cassava plants compared to that from uninfected plants irrespective of whether the plants were resistant, moderately resistant or susceptible (Table 2). The highest difference of 0.36 was noted for the moderately resistant cultivar as the uninfected plant had a protein percentage of 1.68% and the uninfected 2.04%. There was significant difference in the protein content of tubers from uninfected plants to their corresponding infected plants at P=0.05 (t-test) (Table 2).

Protein composition on fresh weight basis

The infected resistant cassava cultivar TMS 30572 had a decrease of 0.03 in the protein content of its tubers compared to its corresponding uninfected

Cultivar while TMS 4(2) 1425 and Isunikankiyan showed an increase (Table 2)

Generally, there was a significant difference in the protein content of tubers from the uninfected plants when compared to the infected for the three cassava cultivars (t- test).

Effect of African cassaya mosaic virus disease on the sugar content of peeled cassava tubers from uninfected and infected plants

Dry weight basis

The three cassava cultivars showed an increase in sugar content of tubers of infected plants compared to the uninfected ones (Table 3). The susceptible cultivar, Isunkankiyan had the highest increase of 0.22%. This difference in the sugar content was significant also for the other two cultivars as values changed from 2.4 - 2.57% for the resistant and 2.49 - 2.52% for the moderately resistant cultivar.

Fresh weight basis

Irrespective of the cassava cultivar, there was a difference in the sugar content of tubers from the infected plants compared to the uninfected ones (Table 3). This difference was significant in all the cassava cultivars, although a decrease of -0.04 in the sugar content of infected plants was also noted compared to that of the uninfected for TMS 6. The other two cultivars had both an increase of 0.1.

Effect of African cassava mosaic virus disease on the starch content of peeled cassava tubers from uninfected and infected plants

Dry weight basis

The percentage starch in peeled cassava tubers from uninfected and infected plants ranged from 72.4 for the uninfected to 71.91 for the infected plant or the moderately resistant cultivar TMS (2)1425 (Table 4).

There was no significance difference in the % starch content between these two values unlike those of the Cultivars TMS 30572 and Isunikankiyan which had a significant decrease in starch content of tubers from infected plants compared to that of the uninfected.

Fresh weight basis

Generally, there was an increase in the starch content of tubers of the infected plants over those of uninfected irrespective of whether plants were resistant, moderately resistant or susceptible (Table 4). There was however no significant difference (P= 0.05) in the starch content of tubers from uninfected and infected pants of TMS 4(2)1425. Significant differences were noted for the resistant and the susceptible cultivars.

Effect of African cassava mosaic virus disease on the fibre content of peeled cassava tubers from uninfected and infected plants

Dry weight basis

Apart from the local cultivar Isu that showed a significant increase of 0.04 in its fibre content of infected over the uninfected plants, the resistant and moderately resistant cultivars showed a reduction of 0.07 and 0.1 respectively (Table 5). This decrease was significant for the resistant cultivar, but not for the moderately resistant cultivar (Table 5).

Fresh weight basis

Two cultivars showed a reduction in their fibre content as a result of infection (Table 5). These included the resistant cultivar TMS 30572, whose % fibre content decreased from 0.75 for the uninfected plant to 0.65 for the infected ones and Isu with 0.86 to 0.74 for the uninfected and infected plants respectively. (Table 5). The differences in fibre content of tubers on uninfected and infected plants for these three cultivars were significant at P = 0.05, t-test.

Effect of African cassava mosaic virus disease on the fat content of peeled cassava tubers from uninfected and infected plants

Dry weight basis

Both the moderately resistant cultivar TMS 4(2)1425 and the resistant TMS 30512 showed a reduction in fat content as a result of infection (Table 6). The reductions were significant at 5% probability level (t- test) (Table 6). For the other cultivar Isu there was no significant difference in the fat content of tubers from uninfected and infected plants

Fresh weight basis

There was no difference in the fat content of tubers of the uninfected and infected plants of TMS 4(2)1425 (Table 6). However, there were significant differences in the fat content of tubers of the resistant and susceptible cultivars.

Effect of African cassava mosaic virus disease on the moisture content of peeled cassava tubers from uninfected and infected plants

Dry weight basis

Generally, on a dry weight basis, there was a significant difference in the moisture content of tubers of the infected cassava plants compared to that of the uninfected irrespective of the cultivar type (Table 7). There was an increase of 0.40 and 0.25 in the % moisture content of tubers from the infected plants over those from the uninfected in the moderately resistant and susceptible cultivars. There was however, a decrease of 0.24% in the resistant cultivar.

Fresh weight basis

On a fresh weight basis, there was a decrease in the % moisture content of tubers from infected plants irrespective of the cultivar (Table 7).

All the cultivars showed a significant differences in the moisture content of tubers from infected plants compared to the uninfected (Table 7).

Effect of African cassava mosaic virus disease on the dry matter content of peeled cassava tubers from uninfected and infected plants

Dry weight basis

In contrast to the resistant cultivar that showed a significant decrease of -0.49 in % dry matter content (dry weight basis) for the infected plants compared to the uninfected, the moderately resistant and susceptible cultivars showed a significant increase of 0.25 and 0.25% respectively (Table 8).

Fresh weight basis

On a fresh weight basis, there was little difference in the dry matter content of tubers from the moderately

resistant and susceptible cultivars when they are infected and uninfected (Table 8). There was however a significant difference in the dry matter content of tubers from the uninfected plants compared to that of the infected plants of the resistant cultivar.

Effect of African cassava mosaic virus disease on the cyanide content of peeled cassava tubers from the uninfected and infected plants

As a result of processing involved in the determination of the cyanogenic potential of cassava tubers on a dry weight basis, most of the cyanide was lost and as such no measurable quantity was obtained.

Generally, on a fresh weight basis, there was an increase in the cyanide content of tubers from infected plants over that of the uninfected irrespective of the cultivars (Table 9). This increase was not significant in the moderately resistant and susceptible cultivars, but was however, significant for resistant TMS 30572.

Table.1 Monthly means of index of symptom severity (ISS) for three cassava cultivars

Cassava cultivars	Months September	October	November	December	January
TMS 30572 (Resistant)	1.16 ± 0.03a	1.26 ± 0.04a	1.31 ± 0.43a	1.36 ± 0.48a	1.36 ± 0.05a
TMS 4(2) 1425 (Moderately resistant)	1.12 ± 0.01a	1.30 ± 0.04a	1.34 ± 0.04a	1.43 ± 0.06a	1.45 ± 0.07b
Isunkankiyan (Susceptible)	2.58 ± 0.19b	2.96 ± 0.17b	3.19 ± 0.22b	3.19 ± 0.08b	3.57 ± 0.01b

-Values are means (± standard error) of two readings per month
-Means followed by the same letter in each column are not significantly different at 3 level of significance (Duncan Multiple Range Test)

Table.2 Effect of African Cassava Mosaic Virus disease on the protein content of peeled cassava tubers from uninfected and infected plants.

Cassava cultivars	Protein Content (%)							
	Dry weight basis				Fresh weight basis			
	Uninfected	Infected	Difference	% Difference	Uninfected	Infected	Difference	% Difference
TMS 30572 (Resistant)	1.72	1.89 ± 0.06*	0.17	9.89	1.12	1.09 ± 0.01**	-0.03	2.68
TMS 4(2) 1425 (Moderately resistant)	1.68	2.04 ± 0.18	0.36	21.43	1.08	1.09± 0.02*	0.01	0.93
Isunkankiyan (Susceptible)	1.63	1.90 ± 0.29*	0.29	16.56	1.04	1.07 ± 0.01*	0.03	2.89

- Values are means of four determinations per cultivar
- Means followed by an asterisk (*) in row are significantly different at 5% level of significance (t-test)

Table.3 Effect of African Cassava Mosaic virus disease on the sugar content ot peeled cassava tubers from uninfected and infected plants

Cassava cultivars	Sugar Content (%)							
	Dry weight basis				Fresh weight basis			
	Uninfected	Infected	Difference	% Difference	Uninfected	Infected	Difference	% Difference
TMS 30572 (Resistant)	2.40	2.57 ± 0.21*	0.17	6.67	5.14	5.15 ± 0.03*	0.01	0.19
TMS 4(2) 1425 (Moderately resistant)	2.49	2.52 ± 0.17*	0.03	1.2	5.18	5.14± 0.02*	0.04	0.78
Isunkankiyan (Susceptible)	2.31	2.53 ± 0.05*	0.22	9.5	5.09	5.19 ± 0.51*	0.1	1.97

- Values are means (± standard error) of four determinations per cultivar:
- Means followed by an astarisk (*) in each row are significantly different at 5% level of significance (t-test).

Table.4 Effect of African Cassava mosaic virus disease on the starch content of peeled cassava tubers from uninfected and infected plants

Cassava cultivars	Starch Content (%)							
	Dry weight basis				Fresh weight basis			
	Uninfected	Infected	Difference	% Difference	Uninfected	Infected	Difference	% Difference
TMS 30572 (Resistant)	72.80	71.03 ± 0.12*	1.77	2.43	36.1	36.46 ± 0.43*	0.36	0.1
TMS 4(2) 1425 (Moderately resistant)	71.54	71.91 ± 0.40	0.37	0.52	36.3	36.38± 0.19*	0.08	0.22
Isunkankiyan (Susceptible)	73.23	72.19 ± 0.66*	0.04	1.42	36.1	36.55 ± 0.25*	0.45	1.25

- Values are means (± standard error) of four determinations per cultivar
- Means followed by an asterisk (*) in each row are significantly different at 5% level of significance (t-test)

Table.5 Effect of African Cassava Mosaic Virus disease on the fiber content of peeled cassava tubers from uninfected and infected plants.

Cassava cultivars	Fiber Content (%)							
	Dry weight basis				Fresh weight basis			
	Uninfected	Infected	Difference	% Difference	Uninfected	Infected	Difference	% Difference
TMS 30572 (Resistant)	1.61	1.54 ± 0.01*	-0.07	4.35	0.75	0.65 ± 0.03*	-0.1	13.3
TMS 4(2) 1425 (Moderately resistant)	1.71	1.61 ± 0.03	-0.1	5.85	0.65	0.77 ± 0.04*	0.12	18.46
Isunkankiyan (Susceptible)	1.57	1.61 ± 0.04*	0.04	2.55	0.86	0.74 ± 0.03*	-0.12	13.95

- Values are means (± standard error) of four determinations per cultivar
- Means followed by an asterisk (*) in each row are significantly different at 5% level of significance (t-test).

Table.6 Effect of African Cassava mosaic virus disease on the fat content of peeled cassava tubers from uninfected and infected plants

Cassava cultivars	Fat Content (%)							
	Dry weight basis				Fresh weight basis			
	Uninfected	Infected	Difference	% Difference	Uninfected	Infected	Difference	% Difference
TMS 30572 (Resistant)	1.86	1.14 ± 0.34*	-0.72	38.71	0.28	0.3 ± 0.01*	0.02	7.14
TMS 4(2) 1425 (Moderately resistant)	1.31	1.10 ± 0.51*	-0.21	16.03	0.30	0.3 ± 0.02	0.00	0.00
Isunkankiyan (Susceptible)	0.87	0.93 ± 0.27	0.06	6.90	0.31	0.29 ± 0.003*	-0.02	6.45

- Values are means (± standard error) of four determinations per cultivar.
- Means followed by an asterisk (*) in each row are significantly different at 5% level of significance (t-test)

Table.7 Effect of African Cassava Mosaic Virus disease on the moisture content of peeled cassava tubers from uninfected and infected plants

Cassava cultivars	Moisture Content (%)							
	Dry weight basis				Fresh weight basis			
	Uninfected	Infected	Difference	% Difference	Uninfected	Infected	Difference	% Difference
TMS 30572 (Resistant)	7.21	6.97 ± 0.22*	0.24	3.33	59.10	58.43 ± 0.21*	-0.67	1.13
TMS 4(2) 1425 (Moderately resistant)	6.33	6.73 ± 0.01*	0.40	6.32	58.80	58.43 ± 0.17*	-0.37	0.63
Isunkankiyan (Susceptible)	93.43	6.82 ± 0.16*	0.25	3.81	59.00	58.45 ± 0.27	-0.55	0.93

- Values are means (± standard error) of four determinations per cultivar.
- Means followed by an asterisk (*) in each row are significantly different at 5% level of significance (t-test)

Table.8 Effect of African Cassava Mosaic Virus disease on the dry matter content of peeled cassava tubers from uninfected and infected plants

Cassava cultivars	Dry matter Content (%)							
	Dry weight basis				Fresh weight basis			
	Uninfected	Infected	Difference	% Difference	Uninfected	Infected	Difference	% Difference
TMS 30572 (Resistant)	92.79	93.28 ± 0.13	-0.49	0.53	40.90	41.58 ± 0.21*	0.68	1.66
TMS 4(2) 1425 (Moderately resistant)	93.43	93.18 ± 0.16*	-0.25	0.28	41.20	41.88 ± 4.24	0.68	1.65
Isunkankiyan (Susceptible)	93.43	93.17 ± 0.16*	-0.26	0.28	41.00	41.55 ± 0.27	0.55	1.34

- Values are means (± standard error) of four determinations per cultivar.
- Means followed by an asterisk (*) in each row are significantly different at 5% level of significance (t-test)

Table.9 Effect of African Cassava Mosaic Virus disease on the cyanide content of peeled cassava tubers from uninfected and infected plants

Cassava cultivars	Cyanide Content (Mg HCN/100g)			
	Fresh weight basis			
	Uninfected	Infected	Difference	% Difference
TMS 30572 (Resistant)	0.79	6.98 ± 0.52*	6.19	78.3
TMS 4(2) 1425 (Moderately resistant)	5.04	16.26 ± 8.91	11.22	22.2
Isunkankiyan (Susceptible)	1.14	5.37 ± 2.95	4.23	37.1

- Values are means (± standard error) of four determinations per cultivar.
- Means followed by an asterisk (*) in each row are significantly different at 5% level of significance (t-test)

Table.10 Correlation between the index of symptom severity of African cassava mosaic virus disease and the nutritional components of cassava tuber on dry weight basis.

	ISS	Protein	Sugar	Starch	Fiber	Fat	Moisture content	Dry matter
ISS								
Protein	0.0175*							
Sugar	0.0041*	0.5557						
Starch	0.1472	0.4839	0.0001**					
Fiber	0.2918	0.9167	0.2508	0.5584				
Fat	0.1965	0.5900	0.8854	0.7833	0.6640			
Moisture content	0.3551	0.1986	0.6767	0.7170	0.0713	0.0373*		
Dry matter	0.5140	0.2822	0.8182	0.3396	0.1520	0.0090**	0.0001**	-

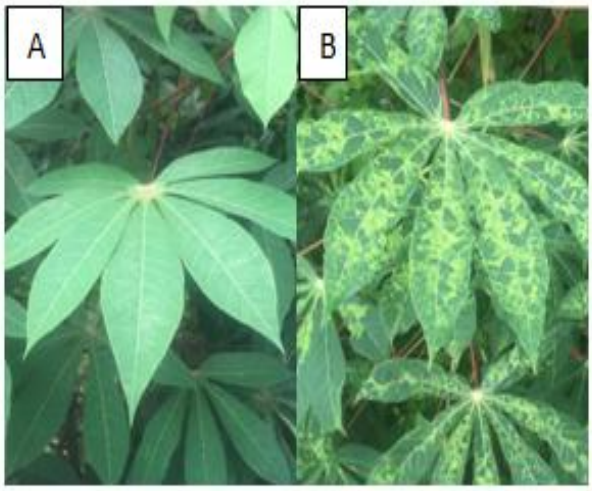
ISS = Index of symptom severity
* = Significant at P = 0.05
** = Significant at P = 0.05 and 0.01

Table.11 Correlation between the index of symptom severity of African cassava mosaic virus disease and the nutritional components of cassava tuber on fresh weight basis.

	MSEV	Protein	Sugar	Starch	Fiber	Fat	Moisture content	Dry matter	Cyanide
MSEV									
Protein	0.6714								
Sugar	0.1453	0.9099							
Starch	0.0164*	0.5525	0.6480						
Fiber	0.5630	0.0335*	0.0338*	0.2128					
Fat	0.2562	0.0621	0.0193*	0.1690	0.5872				
Moisture content	0.0045**	0.7753	0.6016	0.0182*	0.0323*	0.5682			
Dry matter	0.6236	0.4778	0.4360	0.3888	0.5401	0.3046	0.4411		
Cyanide	0.3041	0.5139	0.5139	0.0663	0.0663	0.5172	0.3234	0.0001**	-

ISS = Index of symptom severity
* = Significant at P = 0.05
** = Significant at P = 0.05 and 0.01

Plate.1 A- Apparently healthy cassava leaf B- Cassava leaf infected with ACMV



Correlation between index of symptom severity and the nutritional components of cassava tuber

There was a positive correlation between index of symptom severity (ISS) and the protein, sugar and moisture contents of cassava tubers although these were not significant P < 0.05 (Table 10). The ISS and starch, fiber, fat and dry matter contents were significantly negatively correlated on dry weight basis. On a fresh weight basis, the sugar, starch, dry matter and cyanide contents showed a non-significant positive correlation with the ISS (Table 11). However, the protein, fiber, fat and moisture contents were significantly negatively correlated with the ISS at P <0.05.

The discussion of this study includes, the symptoms of ACMV observed on cassava have similarly been

observed on cassava leaves by Onwueme and Charles (1994). They noted that the most prominent symptom of ACMV infection is chlorosis of discrete areas of the leaf lamina, leaf area reduction and twisted or distorted leaves. The pathogen responsible for cassava mosaic disease in Africa is the *African cassava mosaic virus* (Beck, 1980). This has been confirmed by several authors (Thresh and Cooter, 2005)

Higher protein content was observed in cassava tubers from infected plants than the uninfected ones on a dry weight basis. This is in line with earlier respects (Oba *et al.,* 1992). The increase may be due to stimulation of protein synthesis as a result of metabolism in response to virus infection. Goodman *et al.,* (1986) observed that the general effect of virus infection on the nutritional components of crops included a decrease in the protein content of crops. This study also supports this observation as a decrease of 2.68% was noted for the protein content of tubers from the infected plants compared to that of the uninfected. This decrease probably occurred as a result of the redirection of host protein synthesis to accommodate the synthesis of large qualities of capsid protein for the replication of the virus.

The sugar content of cassava tubers from infected plants increased generally, over that of uninfected plants in the three cassava cultivars. This is in agreement with Alagianagalingam and Ranakrishnam (1970). The increase may be due to an increase in the activities of amylase enzymes in cassava tubers as a result of virus infection (Fraser, 1997).The amylase enzyme acts on starch and degrades it to a simple sugar called amylose. Goodman *et al.,* (1986) also reported an increase in the sugar content of crops infected with virus diseases.

There was high starch content (73.28%) in the tubers of susceptible cassava plants. This is in line with reports IITA (1990a) and FAO (2010). There was however, a reduction in the starch content of cassava tubers in infected resistant and susceptible cultivars as previously noted by Tiwari *et al.,* (2023) and Gulnazym *et al.,* (2023). The reduction may be as a result of decrease in the rate of photosynthesis or decrease in the rate at which starch is translocated from the leaves at night.

Carr *et al.,* (1984) however obtained contrary results in which he noted an increase in the starch content of crops infected by viruses. There was a reduction in the fiber content of cassava tubers as a result of ACMD infection in the resistant and moderately resistant cassava cultivars, but not in the susceptible cultivar.

Also, there was a decrease in the fat content of tubers from infected cassava plants when compared to the uninfected ones for the resistant and moderately resistant cultivars on a dry weight basis. Narasimhan and Arjunan (1973) obtained comparable results. Carr (1984), however, noted an increase in the fat content of crops infected by viruses. Decrease in the fat content of tubers from virus infected cassava plants may be due to degeneration of chloroplast (Narasimhan and Arjunan, 1973). All the three cassava cultivars showed a decrease in dry matter content of their tubers as a result of ACMD infection on a dry weight basis. Gulnazym *et al.,* (2023) made as well a similar observation with bean common mosaic virus in beans.

In contrast, all the three cultivars showed an increase in the dry matter content as a result of infection on a fresh weight basis. Carr (1984) had similar results.

Cyanide content of tubers from infected plants was far higher than that of tubers from uninfected plants. In fact an increase of 78.3% was noted for the resistant cultivar (Table 9).

The positive correlation between virus infection and protein, sugar and moisture content was an indication that an increase in the severity of infection will cause a Corresponding increase in these nutritional components.

In summary, ACMD causes chlorosis of discrete areas of leaf lamina, twisted and distorted leaves that fail to expand fully and also a reduction in size of

the petiole. African cassava mosaic virus disease is the major cause of changes in the nutritional components of cassava tubers.

Within the limitation of this experiment, ACMD caused an increase in the sugar and cyanide contents of cassava tubers and a decrease in the fat and fiber contents. However, for the protein, starch, moisture and dry matter contents, their compositions were more varied and less predictable. The effect of ACMD was greatest on the cyanide content, the most important anti-nutritional component of cassava tuber.

Due to the high increase in the cyanide content of cassava tubers from infected plants, their tubers remain a nutritional threat to man, animals, birds and the environment. Therefore, more studies are needed to breed and develop cassava cultivars in which virus infection would not adversely affect the nutritional components of plants.

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