EFFECTS OF TWO NIGERIAN STRAINS OF *MOROCCAN WATERMELON MOSAIC VIRUS* (MWMV) ON ELEMENTAL AND PROXIMATE COMPOSITION OF THE LEAVES OF *CUCUMEROPSIS MANNII* NAUDIN

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SUMMARY

The effects of two Nigerian strains of *Moroccan watermelon mosaic virus* (MWMV), (designated as MWMV-cor and MWMV-lag) on the elemental and proximate composition of the leaves of *Cucumeropsis mannii* were studied. Infected and healthy (control) leaf samples were obtained, pulverized and analyzed for elemental and proximate composition using standard methods described by AOAC. Inoculation with MWMV-cor led to significant increase ($p<0.05$) in Pb, Co, Fe and Ca compared to healthy sample by 64.03%, 18.18%, 12.12% and 11.99% respectively. Values obtained for Zn, Al and Mg, though higher than the healthy control, were statistically insignificant. Conversely, MWMV-cor engendered significant reduction in Cu content by 17.48%, Cd by 53.13% and Ni by 60.61% while values obtained for Mn, Na and K were comparable to the value obtained for healthy control and consequently not significantly different ($p<0.05$). For MWMV-lag, there were significant increases in Pb content by 39.07%, Cu by 19.54%, Co by 45.45%, Ni by 38.46% and Na by 12.18%. Values obtained for Ca, Zn and Mg, though higher, did not differ significantly ($p<0.05$) compared to healthy sample. On the other hand, Cd and Mn were significantly reduced by 50% and 12.41% by the virus strain while the reduction in Al and K were statistically insignificant. The content of Fe remained unaltered by MWMV-lag infection. With regard to proximate composition, there was an increase in the moisture and fibre contents of *C. mannii* when inoculated with MWMV-cor compared to healthy control while there were reductions in the amount of protein and lipid in infected leaf tissues. Inoculation of the

crop with MWMV-lag led to significantly higher protein content (33.62%) in infected leaf tissue compared with control. Conversely, the virus strain caused insignificant reduction in the moisture, lipid and carbohydrate contents in relation to the control. The implication of this study is that an increase or decrease in the mineral and proximate components of infected *C. mannii* by MWMV strains is an indication of altered physiology of the crop and could impact negatively on the yield performance and a lowering of potentials of seeds from such harvest for industrial use.

Keywords: *Cucumeropsis mannii*, *Moroccan watermelon mosaic virus* (MWMV), elemental and proximate composition.

WHITE-SEED MELON (*Cucumeropsis mannii* (Nudin) (syn = *C. edulis*), is indigenous to West and Central Africa. A member of the family Cucurbitaceae, it is a monoecious scandent herb and can grow up to 5-10 m long, climbing by simple tendrils. The stem is angular and sparsely hairy. The heart-shaped or roughly palmate leaves are up to 12 cm long and 14 cm wide. The seeds, which are milky in colour, are ovate in shape, compressed and smooth (1).

In sub-sahara Africa, C. mannii is priced for its oleaginous seeds that together with seeds of Citrullus Schrad., and Cucumis L. species are used to thicken a traditional dish called "egusi-soup" in Cameroon, Nigeria and Benin Republic, and "postachie" in Cote d'Ivoire (2, 3). In Northern Ghana "egusi" oil is the second most prominent cooking oil (4). Most commonly it is dehulled and consumed as a snack (5). The kernel of the egusi-itoo seed contains semi-drying oils which can be used for soap making and for illumination, with the rest of the seed fed to livestock. Another consumption of the melon is in patty form. After oil has been extracted from the seed, it is then eaten as a protein substitute. Most commonly it is dehulled and consumed as a snack (4).

There are several reports of nutritional composition of *C. mannii*. Dehulled seeds from *C. mannii* mainly have been found to consist of 40 - 44% fat/lipids and 34.5 - 43.63% protein. Carbohydrates, minerals and water amount to 16.5, 3.7, and 5.9%, respectively (6, 7). The oil of the seed has been reported to contain between 62.42 - 64.9% linoleic acid, 12.4 -15.9% oleic acid, 11.8% stearic acid and 10.27 -10.9% palmitic acid (6). Vitamins, thiamin, niacin, B1 and B2 are also prevalent in the seed (4). Notable minerals include phosphorus as the largest

mineral component, with potassium, magnesium, manganese, sulphur, calcium, iron and zinc in that order. The bulk of carbohydrates are starch and soluble sugars (1, 4**).** Egusi-itoo has been considered as the perfect complement to the largely starch-rich grain diet of Africa, providing a high-protein and high-energy concentrate. The seed is considered an excellent vegetable protein, and ideal for battling nutritional debilitations (4, 6). Just 100 g of seed daily is considered enough to provide essential fatty acid, amino acid and Vitamin E requirements (5).

Seed oil metallic soaps derived from *C. mannii*, when incorporated into paint matrix has been shown to act as catalyst, reducing the drying time (8). The potential use of the seeds as raw material for biodiesel production has also been demonstrated, as the measured fuel properties of the fatty acid methyl ester of the oil were found comparable to both the ASTMD 6751 and the EN 14214 biodiesel standards (9). The oil from the seeds has been established to be better than castor oil in methyl salicylate liniment and salycilic acid lotion formulation (10). The seeds of the crop have also been found to contain inhibitory effects on key enzymes relevant to erectile dysfunction and the used of the seeds to manage this condition has been recommended (11).

Moroccan watermelon mosaic virus (MWMV) belongs to the genus *Potyvirus* (family: *Potyviridae*) and is characterized by flexuous particles of about 730 nm in length. The virus which was first reported in Morocco (12), causing severe diseases characterized by mosaic, leaf malformation, green-vein banding, and stunting in several susceptible cucurbits, has been reported from southwest Spain (13) and Italy (14). In Africa, the virus has been reported in South Africa (15), Sudan (16), Democratic Republic of Congo (17), Tunisia (18) and in Nigeria, where two strains of the virus have been identified (19). The virus is now considered an emerging threat to commercial production of cucurbits where ever it is found.

Changes in the elemental composition of virus infected plants have been documented in literature. Shattuck (20) reported that infection of rutabaga (*Brassica napus* sp. *rapifera* inoculated at maturity with *Turnip yellow mosaic virus* (TuMV) led to higher N, P, Mg but a lower K content in infected leaf tissues compared to healthy controls. Noqueira *et al* (21) reported low levels of N and higher levels of Ca, S, and Fe as compared to the control in *Citrus sinensis* inoculated with

Citrus leprosis virus (CiLV) while the values obtained for P, K, Mg, Cu, Mn, Zn and Bo were comparable to healthy. A study on the mineral composition of broad bean (*Vicia fabae*) inoculated with *Broad bean mosaic virus* (BBMV) showed that infected plant contained more P and less total nitrogen and K than in healthy plant sample (22). Muqit *et al* (23) reported reduction in the amount of N in ash gourd (*Benincasa hispidia*) due to infection by *Bottle gourd mosaic virus* (BGMV). The study by Yardimci *et al* (24) revealed that *Alfalfa mosaic virus* (AMV) caused reduction in P, Fe, Cu, Zn and Mn and increased N content compared to healthy plant samples of alfalfa. Owolabi *et al* (25) reported significant reductions in Mg, Fe and Ca contents with increases in P, Mn and K in Ivy gourd leaves (*Coccinia barteri*) infected with a Nigerian strain of MWMV. According to Shakeel *et al* (26), there was an increase in Mg content, a significant reduction in K content while Na content was either reduced or increased in cucumber, depending on the varieties when inoculated with *Cucumber mosaic virus* (CMV).

Plant virus infection has also been reported to alter proximate composition of infected plants. Nambiar and Ramakrishnan (27) have reported reduction in the carbohydrate level in pigeon pea infected with *Pigeon pea sterility mosaic virus* (PPSMV). Reduction in the carbohydrate content of infected leaves of papaya inoculated with *Papaya ringspot virus* (PRSV) was reported by (28). Sinha and Srivastava (29) have also reported lower carbohydrate content but increased protein content in mungbean plants inoculated with *Mungbean yellow mosaic virus* (MYMV). Cheema *et al.* (30) showed that protein contents in two soybean varieties increased when infected by *Soybean yellow mosaic virus* (SYMV). Mofunanya *et al* (31) have documented reductions in protein, fibre, carbohydrate, lipids and ash contents in *Telfairia occidentalis* inoculated with *Telfairia mosaic virus* (TeMV). Samples of bean plant (*Phaseolus vulgaris*) and sugar beet (*Beta vulgaris* L.) inoculated with *Bean common mosaic virus* (BCMV) were reported to contain more protein than healthy one (32,33).

Cucumeropsis mannii is an important vegetable crop in the Southwest and northern parst of Cross River State of Nigeria. It has been used as experimental host for the two strains of MWMV in our previous studies (29,34) in which they induced severe mosaic, severe leaf malformation, reduced leaf size, severe stunting,

sometimes culminating in growth cessation**.** The crop has indeed become the choice host for isolating cucurbits viruses in our researches. The focus of this study is to examine the effects of the two Nigerian strains of MWMV-cor and MWMV-lag on the biochemical and physiological changes in this economic plant with respect to mineral and proximate composition.

MATERIALS AND METHODS

Source of Seeds

Seeds of *C. mannii* used in this study were sourced from Ogoja in Cross River State, Nigeria. Authentication of seeds was carried out by Mr. Frank I. Apejoye (a taxonomist) in the Department of Botany, University of Calabar, Calabar, Nigeria.

Strains of virus sources and preparation of virus inocula

The two virus strains designated as MWMV-cor and MWMV-lag in this research were the isolates described by Owolabi *et al* (19), isolated from *Coccinia barteri* (Benth.) Roberty and *Lagenaria breviflora* (Hork. F) Kay respectively. The viruses were propagated and maintained on *C mannii* in the screen house of the Department of Botany, University of Calabar, Calabar. Virus inocula were prepared by grinding symptomatic leaves in 0.5 M potassium phosphate buffer, pH 7.5, in sterile pestles and mortars.

Experimental design and inoculation of experimental plants

Seeds were sown in perforated polyethylene bags (16 cm diameter), each filled with about 4.2 kg of heat-sterilized loamy soil. The experiment was laid out in complete randomized design consisting of 4 replications. Each replication contained 24 seedlings, arranged in 8 rows and 3 columns. Prior to inoculation, the surface of the leaves was dusted with carborundum (abrasive of 6000 mesh). Using a table of random numbers, the inocula (MWMV-cor, MWMV-lag and control which was the buffer only) were randomly applied separately on the plants within each replication by mechanical or sap inoculation in the screen house with average temperature of 23 ± 3^0C. The inoculated plants were rinsed with distilled water and left for symptom development.

Preparation of sample before analysis

Four weeks after inoculation when symptoms on the leaves had become severe, the infected leaves were harvested, rinsed with distilled water, shredded and oven-dried at 70^0C to constant weight and pulverized in an

electric mill (National Food Grinder, Model 2008 Mk, Japan). Healthy leaf samples were similarly treated as described for infected samples. One hundred grams (100 g) were taken from the pulverized samples for elemental and proximate content determinations.

Digestion of samples for determination of minerals

Digestion of samples was carried out using the dry digestion method as described by AOAC (35). Two (2) g each of the powered samples was weighed into a crucible and swirled gently to mix the content. Each sample was leached with 5 ml of 20% HCl and subsequently transferred into 20 ml volumetric flask. The volume was made up to 20 ml with distilled water. A blank solution was also prepared in a similar way except for the omission of the samples.

Determination of mineral contents

The contents of Al, Cd, Co, Cu, Fe, Mn, Ni, Pb, Zn, Ca and Mg were determined using an atomic absorption spectrophotometer (Pye Unical SP8, Spec. UK) as outlined in AOAC (36). To 2 g sample was added 20 ml of acid mixture (65 ml conc. HNO_3, perchloric acid and 2 ml conc. H_2SO_4) in a digestion flask, heated gently at between 50-70^{O}C on a Stuart hot plate until clear digest was obtained and made up to 100 ml with deionized water. Appropriate dilutions (2.0 ppm, 4.0 ppm, 6.0 ppm, 8.0 ppm and 10.0 ppm) were made for each element. For Ca and Mg determination, strontium chloride ($SrCl_2$) was added to yield a 1500 mg/ml of Sr^{2+} in the final solution. Calibration curves were prepared for each element. The contents of the elements were determined using the calibration curves drawn from data obtained spectrophotometrically using the appropriate wavelengths for each of the elements.

Na and K contents were estimated by flame photometry (Perkin Elmer Analyzer 2880 Spain). The digestion procedure was as described for atomic absorption spectrophotometry. The stock solutions for Na (1000 ppm Na^{+}) and K (1000 ppm K^{+}) were prepared by dissolving 2.542 g of NaCl and 1.907 g of KCl. Working standards (2.0 ppm, 4.0 ppm, 6.0 ppm, 8.0ppm and 10.0 ppm) from the stock solutions were also prepared. The absorbances for Na (at 589 nm) and K (at 767 nm) were obtained using the flame photometer. Na and K concentrations were determined from the calibration curves obtained from the working standards solutions and the results were expressed in milligram per litre.

The phosphorus content of the samples was determined as described in AOAC (36). To 0.5 ml aliquot of the mineral digest was added 9.5 ml of 10% trichloroacetic acid in a 16 x 25 mm test tube. The mixture was agitated, centrifuged (Censaur 2 MSE, UK) for 5 min and then filtered through Whatman filter paper. Five (5) millilitres of the filtrate and 5 ml of the working standards prepared as previously described from P stock solution (4.324 g KH_2PO_4 in 250 ml of deionized water and made up to 1 litre) were measured into two 19 mm cuvettes to which 0.5 ml of molybdate reagent (prepared by adding 200 ml diluted water to 83 ml of conc. H_2SO_4 and 25 g ammonium molybdate tetrahydrate made up to a litre by the addition of deionized water). The cuvettes were shaken and 0.2 ml sulphuric acid reagent (0.125 g 1,2,4-aminonaphthosulphuric acid, 7.28 g sodium bisulphite and 0.25 g sodium sulphite in 50 ml of distilled water) was added, stoppered, shaken and allowed to stand for 10 min. The absorbance of the test and standards were read at 660 nm in the spectrophotometer. Phosphorus concentration was obtained from calibration curve of the standards.

Determination of moisture content

For the moisture content determination, a 3 g sample of infected and non-infected leaves were separately weighed into porcelain dishes and covered with lids. The dishes and contents were placed in cooled desiccators containing con. H_2SO_4 as drying agent and then weighed. The procedures were repeated until constant weights were obtained for each sample as expressed in the relation below:

$$\% \text{ moisture} = \frac{\text{loss in weight on drying (g)}}{\text{Initial weight of sample (g)}} \times 100$$

Determination of the crude protein

The proximate contents (crude protein, ash, lipid, fibre moisture and carbohydrate) of the samples were determined by the methods of the Association of Official Analytical Chemists (AOAC) (36). The crude protein content was determined by the Kjeldah method. Two (2) gram sample was introduced into the Kjeldahl flask held at an angle to which 20 ml of conc. sulphuric acid was added. The flask was placed in the stand and heated over a gentle flame until the initial charring subsided after which 2 Kjeldahl C

(5.00 g K_2SO_4 and 0.100 g Cu $SO_4.5H_2O$) was added to the flask by sliding them carefully down the neck of the inclined flask. The content of the flask was further heated gently at first and then more vigorously until the solution turned green. It was then allowed to cool to room temperature. Thereafter, 2000 ml of distilled water was added and the flask swirled carefully to mix the content. The flask was connected to the distillation apparatus, ensuring that the delivery tube dipped below the surface of 50 ml boric acid solution (prepared by dissolving 40 g of boric acid in 800 ml of distilled water and made up to 1 litre by adding water). To this was added 2-3 drops of silicon antifoam reagent followed by 80 ml NaOH solution (1 g NaOH in 1 litre of water) and the distillation apparatus gently agitated to ensure thorough mixing. The flask was then heated. About 160 ml of distillate was obtained and this was titrated using 0.1N sulphuric acid to the blue grey end point (pH 4.5) of the indicator solution (prepared by dissolving 0.16 g methyl red and 0.083 g bromocresol green in 100 ml industrial methylated spirit). A blank experiment (without sample) was carried out in a similar manner and the titre from it was subtracted from that containing the sample to obtain the true titre. Percentage N in the sample was obtained using the formula:

$$N\% = (T - B) \times 0.0014 \times 100/M$$

Where T = ml of 0.1N sulphuric acid for sample

B = ml of 0.1N sulphuric acid for blank

M = weight of sample (g)

The crude protein content was then calculated by multiplying the N value by the protein factor 6.25.

Determination of lipid content

For the crude lipid determination, the petroleum ether extraction protocol (32,36) was used. Ten-gram of infected and non-infected samples was separately introduced into a fat extractor thimble to which 150 ml petroleum ether (boiling range 40-60 ^{0}C) was added. The thimble with its content was placed into the Soxhlet apparatus (Quickfit, England) to which a pre-weighed round bottom flask had been connected and was heated. The extraction process lasted for 8 h. The flask was disconnected and the solvent distilled off over a steam bath at 50 ^{0}C. The remaining traces of the solvent were removed with a current of air. The flask was dried to constant weight at 100 ^{0}C.

The weight of the crude lipid content was obtained as the difference between the weight of the flask before and after the exaction and evaporation of petroleum ether from the extract.

$$\% \text{ crude lipid} = \frac{\text{weight of flash + sample (g) - wt of flask}}{\text{Weight of sample (g)}}$$

Determination of crude fibre content

For crude fibre determination, two (2) gram defatted sample was weighed and quantitatively transferred into a 400 ml beaker to which 50 ml of 1.25% sulphuric acid (H_2SO_4) was added and the mixture made up to 200 ml with distilled water. This was heated to boiling for 30 min. The content of the beaker was filtered using a Buchner funnel with the aid of a suction pump. The residue was washed with hot water until it was acid free. The residue obtained after the acid digestion was quantitatively transferred into a 400 ml beaker to which 50 ml of 1.25% NaOH was added and made up to 200 ml with distilled water. The mixture was again heated for 30 min with constant stirring. The content of the beaker was filtered through the Buchner funnel and washed five times with water until free of NaOH. The residue obtained was washed twice with 95% methanol and quantitatively transferred into a porcelain crucible and dried at 100 ^{0}C. The weight of the oven-dried residue was noted and later ignited in a furnace at 550 ^{0}C. The weight of the ash left after ignition was also noted. The crude fibre content was determined from the weight loss of the crucible and its content after the ignition.

Determination of ash

For the ash content, 2 g of infected and non-infected samples were weighed separately into dry pre-weighted porcelain crucibles. The crucibles were placed in a muffle furnace (Gallenkamp, Muffle Furnace Size 2, UK) and ignited for 24 h at 550 ^{0}C to eliminate the organic components and until completely ashed to gray white. The crucibles and their contents were cooled in desiccators and later weighed. The difference in weights before and after incineration was reported as the ash content and expressed as percentage thus:

$$\% \text{ ash} = \frac{\text{weight of dish with ash – wt of dish}}{} \text{ x } 100$$

weight of dish and sample – wt of dish

Determination of carbohydrate content

The carbohydrate content in the various samples was carried out by subtracting the combined values of crude protein, fibre, lipid and ash from the total dry matter.

Statistical Analysis

The data obtained were analysed using the Student T-test. Mean values were compared at 5% confidence limit to determine whether differences were significant. Values presented were means of three replicates.

RESULTS

The results of MWMV-cor infection on the mineral content of *C. mannii* are presented in Table 1. The results showed there was a significant increase ($p<0.05$) in Pb, Co, Fe and Ca contents when compared to the corresponding controls. Mean values obtained for these elements were 13.50 ± 0.05, 0.13 ± 0.00, 13.23 ± 0.00 and 825.35 ± 0.66 mg/l while the controls were 8.23 ± 0.01, 0.11 ± 0.00, 11.80 ± 0.00 and 737.00 ± 0.57 mg/l respectively. Percentage increases ranged from 11.99 for Ca to 64.03 for Pb above the values obtained for the controls. Though the values obtained for Zn, Al and Mg were higher compared to the controls, they were however, statistically insignificant ($p<0.05$).

Conversely, the virus strain engendered significant reduction in the amount of Cu, Cd and Ni with mean values of 3.45 ± 0.00, 0.15 ± 0.00 and 0.13 ± 0.01 mg/l respectively compared to the corresponding controls with mean values of 4.18 ± 0.57, 0.32 ± 0.03 and 0.33 ± 0.00 mg/l. Percentage reductions were as high as 53 for Cd and 60.61 for Ni. Values obtained for Mn, Na and K were comparable and consequently not significantly different compared to healthy controls.

Table 2 showed the results of MWMV-lag infection on the mineral composition of *C. mannii*. Except for Cd, Al, Mn, and Fe, there was an increase in the amount of all the other elements in the infected leaf tissue compared to the controls. The contents of Pb, Cu, Co, Ni, and Na (with means values of 11.32 ± 0.05, 5.20 ± 0.01, 0.16 ± 0.00, 0.18 ± 0.01and 1154 ± 0.33 mg/l respectively while the corresponding values for the controls were 8.14 ± 0.13, 4.35 ± 0.30, 0.11 ± 0.00, 0.13 ± 0.00 and 1029.33 ± 0.33 mg/l) were significantly higher ($p<0.05$) in infected leaf tissues compared to the controls. Percentage increases were

as high as 39.7 for Pb, 38.46 for Ni and 45.5 for Co. The values obtained for Ca, Zn and Mg, though higher compared to the corresponding controls did not differ significantly ($p<0.05$). Infection by the virus significantly reduced the amount of Cd and Mn with a mean value of 0.13 ± 0.01 and 7.20 ± 0.01 compared to the controls that had mean values of 0.26 ± 0.00 and 8.22 ± 0.23 with percentage reductions of 50 and 12.41. The reductions caused by the virus in the amount of Al and K were statistically insignificant while the amount of Fe was unaffected by the virus infection.

Table 1: Effect of *Moroccan watermelon mosaic virus* (Coccinia strain) infection on elemental composition of *Cucumeropsis mannii*

Element	Infected samples (mg/l)	Healthy samples (mg/l)	% Diff[a]
Lead (Pb)	13.50 ± 0.05*	8.23 ± 0.01	64.03
Cooper (Cu)	3.45 ± 0.00*	4.18 ± 0.57	17.48
Cobalt (Co)	0.13 ± 0.00*	0.11 ± 0.00	18.18
Cadmium (Cd)	0.15 ± 0.00*	0.32 ± 0.03	53.13
Nickel (Ni)	0.13 ± 0.01*	0.33 ± 0.00	60.61
Iron (Fe)	13.23 ± 0.00*	11.80 ± 0.00	12.12
Calcium (Ca)	825.35 ± 0.66*	737.00 ± 0.57	11.99
Zinc (Zn)	11.25 ± 0.01†	10.50 ± 0.01	7.14
Aluminium (Al)	106.67 ± 0.36†	101.30 ± 0.05	5.30
Manganese (Mn)	8.47 ± 0.01†	8.70 ± 0.15	2.64
Magnesium (Mg)	518.00 ± 0.57†	514.70 ± 0.88	0.64
Sodium (Na)	1015.33 ± 0.66†	1016.33 ± 0.33	0.10
Potassium (K)	1127.66 ± 0.88†	1136.33 ± 0.66	0.76

Values presented were means ± SD of three determinations.

* Significant; † = not significant ($p<0.05$).

a = values were obtained by expressing the difference between the values of the control and the infected as a percentage of the control.

Table 2: Effect of Moroccan watermelon mosaic virus (Lagenaria strain) infection on elemental composition of *Cucumeropsis mannii*

Element	Infected samples (mg/l)	Healthy samples (mg/l)	% Diff[a]
Lead (Pb)	11.32 ± 0.05*	8.14 ± 0.13	39.07
Cooper (Cu)	5.20 ± 0.01*	4.35 ± 0.30	19.54
Cobalt (Co)	0.16 ± 0.00*	0.11 ± 0.00	45.45
Cadmium (Cd)	0.13 ± 0.01*	0.26 ± 0.00	50.00
Nickel (Ni)	0.18 ± 0.01*	0.13 ± 0.00	38.46
Iron (Fe)	11.84 ± 0.01†	11.84 ± 0.02	0.00
Calcium (Ca)	685.67 ± 0.33†	755.70 ± 21.67	9.27
Zinc (Zn)	11.32 ± 0.00†	10.54 ± 0.04	7.40
Aluminium (Al)	94.70 ± 0.05†	100.73 ± 0.68	5.99
Manganese (Mn)	7.20 ± 0.01*	8.22 ± 0.23	12.41
Magnesium (Mg)	536.00 ± 0.04†	513.00 ± 0.97	4.48
Sodium (Na)	1154.66 ± 0.33*	1029.33 ± 0.33	12.18
Potassium (K)	1197.66 ± 0.88†	1137.33 ± 0.66	5.30

Values presented are means ± SD of three determinations,

* Significant; † = not significant ($p<0.05$).

a = values were obtained by expressing the difference between the values of the control and the infected as a percentage of the control.

The results of the effects of MWMV-cor on the proximate composition of *C. mannii* are presented in Fig 1. The results showed increase in the contents of moisture and fibre. The mean values for infected samples were 82.66 ± 0.47 and 3.72 ± 0.00 respectively, while the healthy samples had values of 81.30 ± 0.49 and 3.67 ± 0.01. There were reductions in the protein, lipid and carbohydrate contents of the inoculated plants when compared with buffer inoculated sample. The results of the mean values for the infected samples were 18.96 ± 0.14,

9.09 ± 0.00 and 61.05 ± 0.15 respectively, while the corresponding mean values of 20.28 ± 0.13, 10.90 ± 0.00 and 64.91 ± 0.19 were obtained for the healthy plants. Analysis of data showed that MWMV-cor infection of *C. mannii* caused significant reduction ($p<0.05$) in the content of lipid (16.60 %).

There was a significantly higher protein content (33.62% increase) following MWMV-lag infection of *C. mannii* compared to the corresponding healthy sample (Fig. 2). A mean value of 20.28 ± 0.13 was obtained for the infected sample while that for the healthy sample was 13.46 ± 0.01. The results also indicated that the virus caused insignificant reduction ($p<0.05$) in the levels of moisture (4.24 %) lipid (2.57 %) ash (3.82 %) and carbohydrate (9.79 %).

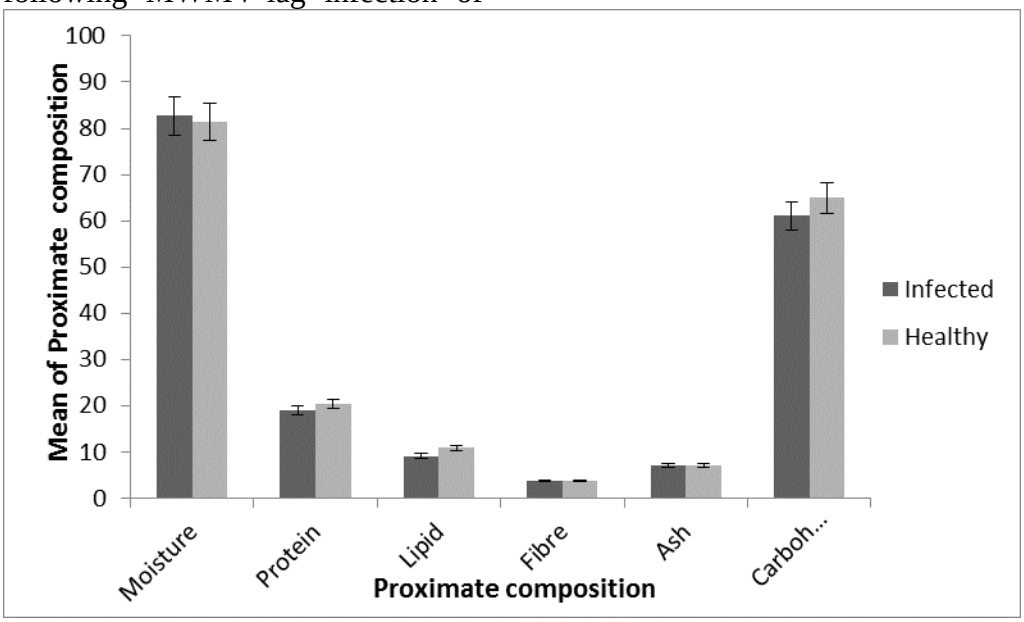

Figure 1: Effect of *Moroccan watermelon mosaic virus* (MWMV-cor) on proximate composition of *Cucumeropsis mannii*

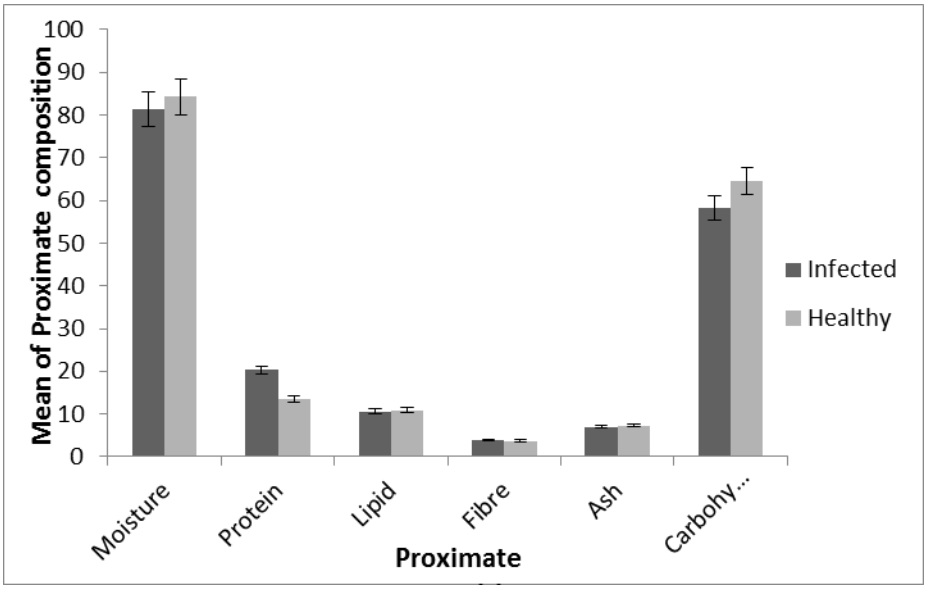

Figure 2: Effect of *Moroccan watermelon mosaic virus* (MWMV-lag) on proximate composition of *Cucumeropsis mannii*

DISCUSSION

The effects of two strains of MWMV (MWMV-cor and MWMV-lag) on elemental and proximate composition of the leaf of *C. mannii* were investigated. In this study, beside Zn and Mg whose contents were found to be higher in plants infected leaf by either of the virus strains, though insignificant, and Cd that was significantly reduced by the virus strains, no general trend could be inferred for the other elements. For instance, there was significant reduction in Pb, Fe, Ca and Co contents in MWMV-cor infected *C. mannii*. Though the values obtained for Zn, Al and Mg were higher compared to the controls, they were statistically insignificant. On the other hand, Cu, Cd and Ni were significantly reduced while the values obtained for Mn, Na and K were not significantly different from compared to the healthy controls. For MWMV-lag, there were significant increases in Pb, Cu, Co, Ni and Na levels in infected leaves while the levels of Zn, K, Mg, though higher, were insignificant compared to the controls. On the other hand, while the contents of Cd and Mn were significantly reduced in infected plants by the virus strains, Ca and Al were higher, though insignificantly, compared to the controls. The level

of Fe apparently was not affected by MWMV-lag infection.

The results of this study seem to confirm previous observations (20, 21, 24, 25) that reactions of plants to virus infection with regard to mineral contents are influenced by plant species or cultivars and virus strains. Comparing their reports with the results obtained in this study, there was also no definitive trend regarding the composition of mineral elements upon virus infection, as reduction in the content of some minerals in one plant-virus combination may be seen to be increased in another plant-virus combination.

Data obtained in this study indicated increase in the moisture and fibre contents and insignificant reduction in the contents of protein, lipid and carbohydrate in MWMV-cor infected leaf tissues of *C. mannii* when compared to healthy controls. The value obtained for ash was comparable with that of the control. Beside protein and fibre whose contents were higher in MWMV-lag inoculated plants, the virus caused reduction in the moisture content as well as lipid, ash and carbohydrate. For moisture content, MWMV-cor infection of *C. mannii* caused increased water content just has been reported for *Tomato aspermy virus* (TAV) infection of tomato (37) while MWMV-lag caused reduction in water content, an observation similar to that of Tinklin (38) in pepper varieties infected with TMV.

The results of this study further confirm previous reports on the fate of protein content in plants following virus infection, which is either an increase or decrease depending on the plant-virus combination. White and Blakke (39) reported increased level of protein in barley infected with WSMV and BSMV separately. Similarly, Cheema *et al* (30) Yardimci (24), Sinha and Srivastava (29) and Kotakadi *et al* (40) recorded higher protein content in soybean, alfalfa, mungbean and sunflower infected with *Soybean mosaic virus* (SoyMV), AMV, *Mungbean yellow mosaic virus* (MYMV) and *Sunflower necrosis virus* (SNV) respectively, similar to the result obtained for MWMV-lag infection of *C. mannii* in this study. Conversely, infection of MWMV-cor caused a reduction in the protein content in the same plant, an observation that had been made in *Benincasa hispidia* infected by *Bottle gourd mosaic virus* (BGMV) (23), *Telfairia occidentalis* (fluted pumpkin) inoculated with *Telfairia mosaic virus* (TeMV) (31) and *Tobacco mosaic virus* (TMV) infection of tomato (41). Potyviruses are known to induce proteinaceous substances

such as pinwheels, scrolls and laminated aggregates in infected plants (16, 42, 43) and these could have been responsible for the increased protein content in MWMV-lag infected *C. mannii*. On the other hand, reduced protein content caused by MWMV-cor may be due to some host gene being shut-off, as suggested by (44).

Reduction in the carbohydrate content recorded in MWMV-cor infection observed in this study is similar to the observation made by Singh (29) in papaya infected with PLRV, Watson and Watson (45) in sugar beet infected *Beet yellow mosaic virus* (BYMV) and Gupta *et al* (46) in soybean (root nodules) infected by *Soybean mosaic virus* (SoyMV). On the other hand, increased carbohydrate content caused by MWMV-lag in this study has similarly been reported in cassava infected by *Cassava brown streak virus* (47). With regard to the lipid content, the present study revealed that infected leaves had less in leaves infected by both strains of MWMV.

The observed decrease in lipid content correlates with those of Bhavani *et al* (**48**) and Kotakadi *et al* (41) who reported lower lipid contents in leaves of sunflower infected by *Sunflower necrosis* virus. The reduced content of lipid could be attributed to lowered synthesis or degradation of lipid.

Mineral elements, particularly micronutrients, are involved in all metabolic and cellular functions and consequently are important in plant growth and development. Their deficiencies or excesses have been reported to impair plant's wellbeing (49, 50, 51). Several of these elements such as boron (B), chloride (Cl), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), nickel (Ni), and zinc (Zn) are essential as catalytically active cofactors in enzymes and have also been reported to modulate the activities of antioxidative enzymes associated with stress **(**52, 53, 54, 55). These they do, according to Bowler *et al* (53) by direct or indirect formation of reactive oxygen species such as superoxide (O^{2-}), hydrogen peroxide (H_2O_2), hydroxyl radical (OH^-) and singlet oxygen, causing lipid peroxidation, protein denaturation and DNA mutation. Others have enzyme-activating functions, and yet others fulfil a structural role in stabilizing proteins (56).

CONCLUSION

From the results of this study, it could be inferred that decrease or increase in the elemental and

proximate contents of *C. mannii* engendered by the MWMV strains**,** were a consequence of perturbation of some physiological processes (since mineral elements and the proximate components are important in biochemical and physiological processes), expressed as visible symptoms such as mosaic, reduced leaf size and severe leaf malformation and stunting of infected plants. Knowing which processes are affected and how these perturbations influence yield performance in this cucurbit needs to be investigated.

ACKNOWLEDGEMENTS

The authors wish to thank Mr. Frank I. Apejoye for identifying the seeds, Mr. Joseph Etim Okon and Emem Okon Mbong for assistance in statistical analysis of data.

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