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Physiological and Biochemical Changes in *Cucurbita moschata* Duch. Ex. Poir Inoculated with a Nigerian Strain of Moroccan Watermelon Mosaic Virus (MWMV): *Lagenaria breviflora* Isolate

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ABSTRACT

This study was carried out to determine physiological and biochemical changes in *C. moschata* inoculated with a Nigerian strain of MWMV isolated from *Lagenaria breviflora* in Calabar, Nigeria. The virus infection resulted in significant ($p \leq 0.05$) reductions in plant height, leaf area and number of leaves produces with highest percentage difference of 39.3% (42 PID), 60.9% (35 PID) and 25.7% (49 PID), respectively. There was a significant ($p \leq 0.05$) difference in chlorophyll content between inoculated and healthy plants. The chlorophyll content of inoculated plants at all stages of growth was lower compared with the healthy plants. Chlorophyll b content was higher than Chl a in both inoculated and healthy plants. Chlorophyll a, b and total Chlorophyll were significantly reduced in inoculated plants when compared with the healthy ones. Of the twelve photochemicals screened, alkaloids, glycosides, saponins, tannins, terpenoids, steroids, polyphenols and reducing sugars were present while phlobatanins, anthraquinones and hydroxymethyl anthraquinones were absent. Moroccan watermelon mosaic virus engendered reductions in alkaloids (32.1%), reducing sugars (42.0%), saponins (33.6%), terpenoids (25.0%) and steroids (15.0%) and increase in inoculated samples of glycosides (14.8%) and flavonoids (12.5%). Effect of MWMV on proximate compositions of *C. moschata* revealed significant ($p \leq 0.05$) reductions in lipid (25.0%) and ash (14.6%) and significant ($p \geq 0.05$) increases in protein (23.4%) and fibre (10.5%). Effect of the virus on carotene, vitamin A and C were insignificant. Infection of MWMV also led to significant decreases in the contents of K (48%), Fe (28.6%), Zn (25.0%) and Mg (22.0%), while Cu recorded 100% increase in inoculated samples. Marginal increase in Ca and P were recorded in inoculated samples. Infection of MWMV on antioxidants caused significant increase in inoculated samples of hydrocyanic acid and insignificant increases in total and soluble oxalate and phytic acid. Increases recorded in the antioxidant contents in plants inoculated with MWMV resulted in reduced bioavailability of minerals in the vegetable. Reduction in chlorophyll contents in inoculated plants would be a major factor explaining poor growth of host plants with attendant reductions in mineral elements of *C. moschata*. Changes in physiological and biochemical parameters induced by MWMV altered vital metabolic processes of *C. moschata*.

Key words: *Cucurbita moschata*, Moroccan watermelon mosaic virus, physiological and biochemical changes, *Lagenaira breviflora*

INTRODUCTION

Cucurbita moschata (Duch ex. Poir) is a member of the family Cucurbitaceae. It is commonly called pumpkin. *Cucurbita moschata* is cultivated as a vegetable crop for its leaves and fruits in

the southern parts of Nigeria, where it also act as effective cover against soil erosion and competes favorably with weeds. The tender leaves of *Cucurbita moschata* are used as vegetables, while the pulp of the slightly unripe fruits is either eaten raw or cooked (Dupriez and De Leener, 1989).

The most common causes of diseases worldwide affecting cucurbits are viruses. A survey of literature on virus diseases of cucurbits revealed that more than 39 well characterized viruses are distributed in unrelated genera, such as *Begomo virus*, *Crinivirus*, *Cucumovirus*, *Polerovirus*, *Tospovirus*, *Ipomovirus*, *Tobamovirus* and *Potyvirus*, which are known to naturally infect cucurbits (Antignus *et al*., 2001; Salem *et al*., 2007; Kneirim *et al*., 2010; Gholamalizadeh *et al*., 2008; Brown *et al*., 2002; Owolabi *et al*., 2008, 2011, 2012).

Moroccan Watermelon Mosaic Virus (MWMV) strain is a *Potyvirus* (family: Potyviridae). The virus has been reported to have caused severe damage to cucurbits in all commercial growing producing regions. Moroccan watermelon mosaic virus is transmitted mechanically by *Myzu persicae* and *Aphis craccivera* with other species of aphids depending on the strain in a non-persistent manner (Polland, 1973). The virus particles are flexus rods about 730-750×10 nm. The virus symptoms in susceptible plant include mosaic, leaf malformation and green banding (Owolabi *et al*., 2008).

Moroccan watermelon mosaic virus was first isolated in morocco (Fischer and Lockhard, 1974). In African countries, the virus has been reported in South Africa (Van Der Meer and Garnett, 1987), Sudan (Lecoq *et al*., 2001), Democratic Republic of Congo (Arocha *et al*., 2008), Tunisia (Yakoubi *et al*., 2008) and Nigeria (Owolabi *et al*., 2012). The virus has also been reported in Spain (Quiot-Douine *et al*., 1990) and Italy (Roggero *et al*., 1998).

Lagenaria breviflora (Benth) Roberty (*Adenopus breviflorus*) belongs to the family Cucurbitaceae. The plant has glabrous stems and leaves which are distinctly 5-lobed with branched tendrils. It is monoecious but the male and female flowers are borne on the same plant separately. The fruits of *L. breviflora* are usually roundish, streaked and flattened at both ends (Burkill, 2004). The seeds of this plant are economically useful and are considered as a good source of amino acids (Oshodi, 1996) and rich in oil (85% unsaturated fatty acid and 65.3% linoleic acid) which could be use in soap making, shampoo, shoe polish and for edible purposes (Akintayo and Bayer, 2002).

Literatures abound on the effect of virus infection on physiological and biochemical changes in plants. El-Dougdoug *et al*. (2007) reported that PVY infection led to a decrease in total alkaloids contents in leaves of *Datura metel*. Duarte *et al*. (2008) also reported a decrease in total phenol and alkaloids content in the same plant inoculated with PVX. Report by Rangaraju and Chenulu (1975) revealed that mosaic virus reduces the fruit content of solasonin (a medically useful alkaloids) to about half. Chong *et al*. (2002) reported that Tobacco Mosaic Virus infection (TMV), inhibited plants. Exhibited a significant decrease in the glucoside form of scopoletin and a decrease in scopoletin UGT activity. Mofunanya and Nta (2011) also reported marked decreases in alkaloids, flavonoid and tannins caused by *Telfariria* mosaic virus and increases in polyphenols and saponins. A decrease in protein, fibre, phosphorus, magnesium, zinc, iron, vitamins and amino acids has been reported by Mofunanya *et al*. (2008) in *Telfairia occidentalis* and in ecotypes of *T. occidentalis* infected with TeMV (Mofunanya *et al*., 2009).

In many host plants infected with different viruses, reduction in chlorophyll content has been reported. In Mungbean plants infected with Mungbena Yellow Mosaic Virus (MYMV), Sinha and Srivastava (2010) reported a reduction in chlorophyll at different stages of growth. Hooks *et al*. (2008) also reported a reduction in chlorophyll content in banana infected with Banana bunchy top virus. In ash gourd, a decrease in chlorophyll content caused by Bottle gourd Mosaic Virus (BgMV), Watermelon Mosaic Virus-2 (WMV-2) and papaya ringspot virus (PRSV) have been reported.

Pazarlar *et al*. (2013) in their work reported a reduction in chlorophyll a and b in some pepper varieties (*Capsicum annuum* L.) infected with Tobacco mosaic virus. Similarly reductions in chlorophyll a, b and total chlorophylls were reported by Mofunanya *et al*. (2014) in ecotypes of *Telfairia occidentalis* infected with *Telfairia* mosaic virus (TeMV).

Mahmood *et al*. (1996) documented that infection of cotton by Cotton leaf curl virus reduced the height of the plant, boil weight, fibre length, fibre strength and fitness due to infection. Significant reduction in plant height has been reported by Taiwo and Akinjogunda (2006) in cowpea viruses, Cowpea Aphid-Borne Mosaic Virus (CABMV), Cowpea Mottle Virus (CMV) and Southern Bean Mosaic Virus (SBMV) in single and mixed viral infection. Work has also been done on the effect of Amaranthus mosaic virus on the growth characters of *Amaranthus hybridus* (Ehinmore and Kareem, 2010). Kareem and Taiwo (2006) observed that cowpea cultivars inoculated with CABMV alone and in combination with SBMV resulted in significant reduction in leaf number when compared with control plants. Infection of banana with banana bunchy top virus showed reduction in petiole size, plant canopy and height, leaf area and pseudostem diameter content when compared with control plants (Hooks *et al*., 2008). Pazarlar *et al*. (2013) also reported significant decrease in growth and physiological parameters in some pepper varieties (*Capsicum annuum* L.) infected with TMV.

Previous studies centered on evaluated of nutritional and phytochemical constituents of *Cucurbita maxima duchesne* and *Cucurbita moschata lam* seeds (Dhanalashmi, 2010). Characterization and identification of a potyvirus causing mosaic disease of *Cucurbita moschata* (Owolabi *et al*., 2011), strains of Moroccan watermelon mosaic virus isolated from *Lagenaria breviflorus* and *Coccinia barteri* (Owolabi *et al*., 2012).

Literatures are lacking on the effects of Moroccan Watermelon Mosaic Virus (MWMV) on growth, chlorophyll and nutritional composition of the vegetable. The present study investigates some physiological and biochemical changes in *Cucurbita moschata* inoculated with a Nigerian strain of MWMV (*Lagenaria breviflorus* isolate).

MATERIALS AND METHODS

Seed collection and planting: Seeds of *Cucurbita moschata* used in this study were obtained from Akparabong in Ikom Local Government Area of Cross River State Nigeria, located on latitude 5°57'N and longitude 8°44'E. The seeds were sown in steam-sterilized soil in 16 cm diameter polyethylene bags and maintained in the greenhouse at 25±3°C.

Virus source and preparation of inoculum: The virus used in the study is a strain of Moroccan Watermelon Mosaic Virus (MWMV) isolated from *Lagenaria breviflorus*. The virus inoculums was prepared by obtaining young symptomatic leaves of *Lagenaria breviflorus*. These leaves were titrated in a pre-sterilized cold mortar and pestle in disodium phosphate (Na_zHPO_4) butter 0.03 M, PH 8.0. The inoculum was applied on leaves dusted with carborundum (800 mesh), rinsed and then left for symptoms development.

Experimental design: Fourty five seedlings of inoculated and healthy plants of *C. moschata* were arranged in three groups each containing fifteen plants each prior to inoculation, the seedling growing in polyethylene bags were arranged in a randomized block design. Within each group nine were inoculated with the virus isolate and the remaining six were inoculated only with the buffer to serve as healthy controls.

Sample preparation for analysis: Two months post-inoculation, inoculated and healthy leaves were harvested randomly from fifteen plants (i.e., from each of the three groups). These leaves were dried in the oven at 65°C and milled into powdered form with the aid of an electric mill (National Food Grinder, Model MK 308, Japan). The powdered samples were then used for the phytochemical, proximate, mineral and antinutrient determination.

Standard procedures were used in the determination of phytochemicals. Alkaloids were determined using, Harbone (1973) method, tannins (Van Buren and Robinson, 1969). Vitamins and minerals were determined by atomic absorption spectrophotometer (Pye Uniam SP8-190, Spec. UK) as outline in AOAC (1995). Phytate was determined according to AOAC (1995), while oxalate content was determined by the method of Dye (1956) as described by Abara *et al*. (2000).

Effect of mwmv infection on growth parameters: Leaf area determination of inoculated (MWMV) and healthy plants of *C. moschata* was carried out at 7 days interval for a period of 56 Post-Inoculation Days (PID). Here inoculated and healthy leaf each was placed on a 1 mm^2 graph paper. The leaf size was traced on the paper and the total area calculated based on the number of squares covered within the traced region.

The formula for leaf area estimation is given as:

A = KLB (cm^2), thus:

$$K = \frac{A}{LB}$$

Where:
A = Leaf area
L = Leaf length
B – Leaf width
K = Correlation coefficient which is constant
(Since, each leaf determination has the development pattern)

Effect of mwmv infection on shoot height and number of leaves: The effect of viral inoculated on short height was determined by measuring stem length in centimeters from soil level to the tip of the stem with the aid of a measuring tape. Measurements were taken as described above. The leaves of both inoculated and healthy plants were counted to determine the effect of the virus infection on the number of produced leaves over the same period of time.

Effect of MWMV infection on total chlorophyll content: Two gram leaf sample obtained from inoculated and healthy plants were used in the estimation of chlorophyll. Measurements were taken at 14 PID intervals. The leaves were grounded using mortar and pestle in 10 mL of 80% acetone. The homogenate were poured into test tubes and centrifuged for 3 min at 4,500 rpm. The supernatants were decanted and then used for chlorophyll estimation. Acetone 3 mL was used to set the blank at zero. To this acetone was added 0.1 mL each of the supernatants from inoculated and healthy samples and absorbance readings taken at wavelength 663 and 643 nm for chlorophyll a and b respectively with the aid of a spectrophotometer (Model 722S, England). Readings were taken from three replicates of each sample and chlorophyll contents were estimated using the formula of Strickland and Parsons (1972):

$$\text{Chl a} = (11.6\ A663 - 1.3\ A643)\ VX^{-1}$$
$$\text{Chl b} = (19.6\ A643 - 4.7\ A663)\ VX^{-1}$$
$$\text{Chl a+b} = (\text{mg g}^{-1}\ \text{FW})$$

where, A663 and A643 represent absorbance at 663 and 643 nm, respectively. A is absorbance, V^{-1} is volume of 80% acetone, X^{-1} is sample fresh weight, mg is milligram, g is gram and FW is fresh weight.

Data analysis: Data obtained in this study were analyzed using the independent t-test. Results were also expressed as percentage difference and difference between mean values were determined at 5% probability.

RESULTS

Results in Table 1 and Fig. 1a, b revealed significant ($p \leq 0.05$) decrease in shoot height, leaf area and number of leaves produced in *C. moschata* infected with Moroccan watermelon mosaic virus. The virus caused progressive decrease in the growth parameters studied with increasing period of development. Shoot height had a range in mean values for inoculated and healthy plants from 9.80±0.62-20.90±0.6 and 9.98±0.62-33.55±0.6 cm corresponding values for leaf area in inoculated and healthy plants ranged from 3.80±-0.3, 25.55±1.8 cm^2 and 3.90±0.3-56.05±1.2 cm^2. While mean values for number of leaves produced in inoculated and health plants ranged from 12.10±0.6-45.02±0.03 and 12.10±0.6-56.05±0.1. Infection of MWMV was insignificant at early period of growth 7 (PID).

Table 1: Effect of MWMV infection on the growth parameters of *Cucurbita moschata*

Post inoculation days	Shoot height (cm)			Leaf area (cm^2)			No. of leaves produced		
	Inoculated	Healthy	%	Inoculated	Healthy	%	Inoculated	Healthy	%
7	9.80±0.62	9.98±0.62	1.80	3.80±0.3	3.90±0.3	2.60	12.10±0.6	12.10±0.6	0.00
14	10.03±0.3	12.83±0.03	21.8	6.10±0.2	8.00±0.1	23.8	22.04±0.1	23.18±0.2	0.05
21	11.05±0.2	15.73±0.5	29.8	8.40±0.4	15.23±0.2	44.8	28.01±0.03	30.12±0.03	7.00
28	12.90±0.3	18.72±0.6	31.1	10.01±0.7	21.83±0.5	54.0	33.19±0.03	40.51±0.6	18.1
35	14.25±0.02	22.05±0.1	35.4	11.85±1.5	30.30±1.2	60.9	36.02±0.6	44.10±	18.3
42	16.33±1.1	26.91±1.1	39.3	15.60±0.23	38.70±0.1	59.7	39.14±0.03	50.08±0.03	19.8
49	18.78±0.3	30.23±0.9	38.0	20.56±3.2	50.30±1.7	59.1	43.11±0.33	55.33±1.2	21.1
56	20.90±0.6	33.55±1.2	37.7	25.55±1.8	56.05±1.2	54.1	45.02±0.03	0.1	20.0

$p \leq 0.05$, values are Mean±SD, N = 3 replicates, Percentage difference was obtained by expressing the difference between the values for inoculated and healthy as a percentage of the healthy

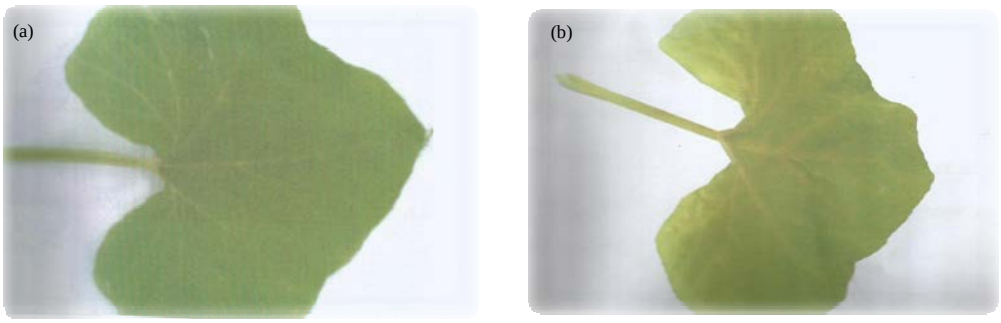

Fig. 1(a-b): Symptoms induced by MWMV on *C. moschata* included mosaic, green vein-banding, rugosity and leaf malformation, (a) Healthy leaf and (b) Inoculated leaf

Table 2: Effect of MWMV infection on chlorophyll content of *Cucurbita moschata*

Post inoculation days	Chlorophyll type	Inoculated (mg g^{-1} FW)	Healthy	%
14	Chl a	30.8±0.02	46.3±0.5	33.5
	Chl b	42.1±0.02	65.6±0.03	35.8
	Chl a+b	72.9	111.9	34.9
	Chl a/b	0.7	0.8	
28	Chl a	35.4±0.04	60.2±0.03	41.2
	Chl b	51.7±0.06	90.5±0.02	42.9
	Chl a+b	87.1	150.7	42.2
	Chl a/b	0.6	0.6	
42	Chl a	42.8±0.03	86.1±0.04	50.3
	Chl b	69.3±0.03	113.4±0.01	38.9
	Chl a+b	1121	199.5	43.8
	Chl a/b	0.6	0.8	
56	Chl a	36.9±0.5	66.2±0.02	44.3
	Chl b	50.5±0.1	96.6±0.06	47.7
	Chl a+b	87.4	162.8	46.3
	Chl a/b	0.6	0.7	

Values are means of three replicates, Percentage difference was obtained by expressing the difference between the values for inoculated and healthy as a percentage of the healthy

Table 3: Qualitative determination of phytochemicals in *Cucurbita moschata* inoculated with MWMV

	Inoculated		Healthy	
Phytochemicals	Ethanol extract	Aqueous extract	Ethanol extract	Aqueous extract
Alkaloids	+	+	++	+
Glycosides	++	+	++	+
Saponins	+	+	+	+
Tannins	+	+	+	+
Flavonoids	++	+	++	+
Terpenoids	+	+	+	+
Steroids	++	+	++	+
Polyphenols	++	+++	++	+++
Reducing sugars	++	+	+++	++
Phlobatanins	-	-	-	-
Anthraquinones	-	-	-	-
Hydroxymethyl Anthraquinones	-	-	-	-

+: Present, -: Absent

Infection of *Cucurbita moschata* with MWMV resulted in significant ($p \leq 0.05$) reduction in chlorophyll a, b and total chlorophyll contents. Higher reduction in chlorophyll content was recorded for chl a when compared with chl b. Lowest and highest mean values for chl a, b and total chl in inoculated plants were 30.8±0.02 and 42.8±0.03, 42.1±0.02 and 69.3±0.03 and 72.9 and 112.1 mg g^{-1} FW, respectively. Corresponding mean values for healthy plants were 46.3±0.5 and 86.1±0.04, 65.6±0.03 and 113.4±0.01 and 111.9 and 199.5 mg g^{-1} FW (Table 2).

In both inoculated and healthy plants, chlorophyll content attained a peak at 42 PID and declined. The result showed that MWMV infection caused significant ($p \leq 0.05$) decrease in the contents of chlorophyll when compared with healthy plants.

Results of qualitative determination of phytochemicals revealed that of the twelve phytochemicals screened in leaves samples of *C. moschata* infected with MWMV, nine (alkaloids, glycosides, saponins, tannins, flavonoids, terpenoids, steroids, polyphenols and reducing sugars) were present and three (phlobatanins, anthraquinones and hydroxymethyl anthraquinones) were absent, glycoside, flavonoids, steroids and reducing sugars were more in ethanolic extract when compared to aqueous, while polyphenols were more in aqueous extract than in ethanolic extract (Table 3).

Effect of MWMV infections on crude phytochemicals (Table 4) resulted in significant ($p \leq 0.05$) reductions in saponins, alkaloids, terpenoids, reducing sugars and steroids with percentage

Table 4: Quantitative determination of phytochemicals in *Cucurbita moschata* Inoculation with MWMV

Phytochemicals	Inoculated	Healthy (mg/100 g)	Difference (%)
Alkaloids	1.97±0.01	2.10±0.01	32.1
Glycosides	2.71±0.01	2.36±0.01	14.8
Saponins	1.46±0.02	2.20±0.01	33.6
Tannins	0.32±0.02	0.31±0.01	3.20
Flavonoids	3.60±0.01	3.20±0.1	12.5
Terpenoids	0.06±0.1	0.08±0.02	25.0
Steroids	0.17±0.02	0.20±0.1	15.0
Polyphenols	2.10±0.1	2.16±0.02	2.80
Reducing sugars	1.12±0.01	1.13±0.1	42.0

$p \leq 0.05$, values are Mean±SD, N = 3 replicates, Percentage difference was obtained by expressing the difference between the value for inoculated and healthy as a percentage of the healthy

Table 5: Effect of MWMV Infection on Proximate Composition of *Cucurbita moschata*

Proximate composition	Inoculated (g/100 g)	Healthy (g/100 g)	Difference (%)
Protein	5.800±0.1	4.70±0.1	23.40
Carbohydrate	79.451±0.02	88.93±0.02	10.70
Lipid	0.630±0.01	0.84±0.01	25.00
Ash	2.820±0.01	3.30±0.1	14.60
Fibre	2.100±0.1	1.90±0.1	10.50
ß-carotene	478.470±0.01	450.73±0.01	6.20
Vitamin A	318.740±0.01	299.70±0.1	6.40
Vitamin C	278.400±0.1	280.30±0.1	0.68

Values are Mean±SD, N = 3 replicates, percentage difference was obtained by expressing the difference between the values for inoculated and healthy as a percentage of the healthy

Table 6: Effect of MWMV Infection on Mineral Composition of *Cucurbita moschata*

Mineral composition	Inoculated (mg/100 g)	Healthy (mg/100 g)	Difference (%)
Sodium	4.89±0.02	5.08±0.01	20
Potassium	3.76±0.03	4.28±0.005	48
Calcium	2.79±0.008	2.77±0.006	2.7
Magnesium	2.22±0.008	2.44±0.41	22
Iron	0.05±0.002	0.07±0.001	28.6
Zinc	0.03±0.001	0.04±0.002	25
Copper	0.02±0.001	0.01±0.001	100
Phosphorus	1.92±0.02	1.86±0.01	3.2

Values are Mean±SD, N = 3 replicates, percentage difference was obtained by expressing the difference between the values for inoculated and healthy as a percentage of the healthy

difference of 33.6, 32.1, 25.0, 42.0 and 15.0%, respectively. Marginal reduction were recorded for tannins (3.2%) and polyphenol (2.8%). Glycosides and flavonoids were noise in inoculated sample when compared to the healthy.

Moroccan watermelon virus infection on the proximate composition of *C. moschata* (Table 5) indicated significant ($p \leq 0.05$) reductions in lipid, ash and carbohydrate contents with percentage difference of 25.0, 14.6 and 10.7%, respectively. Protein and fibre contents were significantly ($p \geq 0.05$) higher in inoculated samples than in healthy ones. Mean values of protein and fibre in healthy samples were 4.70±0.1 and 1.90±0.1 mg/100 g while inoculated samples had values of 5.80±0.1 and 2.10±0.1 mg/100 g. Marginal increases in inoculated samples were recorded for vitamin A, β-carotene and vitamin C.

Results obtained for effect of MWMV on mineral composition showed significant ($p \leq 0.05$) reductions in potassium, iron, zinc, magnesium and sodium with percentage reduction values of 48, 28, 25, 22 and 20%, respectively. Hundred percentage increase in inoculated sample was recorded for cupper while phosphorus and calcium depicted insignificant increases (Table 6).

Antioxidants (hydrocyanic acid, total oxalate, soluble oxalate and phytic acid) were significantly ($p \geq 0.05$) higher in inoculated samples the healthy with percentage difference of 22.1% for Hydrocyanic acid.

Table 7: Effect of MWMV infection on antioxidant in *Cucurbita moschata*

Antioxidant	Inoculated (mg/100 g)	Healthy (mg/100 g)	Difference (%)
Hydrocyanic acid	5.30±0.1	4.34±0.02	22.1
Total oxalate	73.40±0.1	67.83±0.01	8.2
Soluble oxalate	27.13±0.01	25.70±0.01	6.0
Phytic acid	34.60±0.1	31.87±0.01	8.6

Values are Mean±SD, N = 3 replicates, percentage difference was obtained by expressing the difference between the values for inoculated and healthy as a percentage of the healthy

DISCUSSION

This study was conducted to determine physiological and biochemical changes induced by MWMV in *C. moschata*. Results of the study show that MWMV caused considerable changes in the parameters investigated as systematic host of the virus (Table 7). It was well expressed in the reduction in the phytochemcial contents of saponins, alkaloids, terpenoids, reducing sugars and steroids. Reductions engendered by MWMV infection are in agreement with previous reports by El-Dougdoug *et al*. (2007), Duarte *et al*. (2008) and Mofunanya and Nta (2011). Significant increases in glycosides and flavonoids and insignificant increase in tannins in infected samples reported in this study are in line with findings by Uegaki *et al*. (1998), who reported on the accumulation of stress compounds due to virus infection. The accumulation of these chemical constituent in inoculated plants suggest that their synthesis is stimulated by stress caused by virus infection. While reduction in phytochemicals could be due to reduction in plant total fresh weight of leaves and demand for viral components synthesis which could cause the host cells to be in competition with normal biosynthetic pathways for essential precursors.

Significant ($p \leq 0.05$) reductions in proximate and mineral constituents are in the line with reports by Mofunanya *et al*. (2008). Antioxidants content were considerable higher in inoculated samples compared with healthy ones.

The reductions depicted in phytochemicals and mineral nutrients in this study require keen attention because the medicinal values of plants and vegetables are revealed by their phytochemical and other constituents as reported by Huseini *et al*. (2005). Edeoga *et al*. (2005) also documented that the presence of these chemical constituents that produce a definite physiological action in the body reveal it's medicinal value. Leafy vegetables are of great importance to the health of individuals as well as communities. The urban and rural poor depend of vegetables as their main and cheap source of mineral elements (Ajakaiye *et al*., 1995). Vegetables are good sources of both macro and micro nutrients.

Phytochemicals are important to both human and animal as they have been linked with positive effect in health including cancer, high blood pressure, coronary heart disease, diabetic, ulcer, muscular degradation, psychotic disease with multiple actions on human health.

Infection of *C. moschata* by MWMV led to significant reduction in growth characteristics. It is not uncommon for virus infection to have a negative impact on plant especially by limiting their growth characteristics (Miteva *et al*., 2005). One explanation for leaf area decrease in inoculated plants could be due to the reduction in the amount of assimilate availability (Ayres, 1992). There thus appear to be a link between assimilate reduction and plant growths.

Reduction in chlorophyll contents agrees with previous studies by Funayama-Noguchi and Terashima (2006), Pineda *et al*. (2008), Singh and Shuka (2009), Pazarlar *et al*. (2013), Sinha and Srivastava (2010) and Mofunanya *et al*. (2014), who have all reported on reduction in chlorophyll content in many plants infected with different viruses.

The differences in chlorophyll content in inoculated and healthy plants is worthy of note as the leaves of inoculated plants appeared to be mosaic. Leaf chlorophyll content can be directly linked

to stress physiology (Hooks *et al*., 2008). Chlorophyll a and b are the two most important pigments used in plant photosynthetic processes and low levels of these chlorophylls can directly limit photosynthetic activity (Taiz and Zeiger, 1991).

Loss of chlorophyll in infected plants has been attributed to impaired chlorophyll synthesis (Funayama-Noguchi and Terashima, 2006). Goncalves *et al*. (2005) attributed loss in chlorophyll to structural alternations in the photosynthetic apparatus while, Guo *et al*. (2005) attributed loss to reduction in the activities of some enzymes associated with electron transport and photosynthesis. From the above, various factors are associated with loss in chlorophyll in infected leaves. Moroccan watermelon mosaic virus is a potyvirus. *Potyviruses* have been known to cause chloroplast aggregation and resulting in a reduction in their number (Wei *et al*., 2010, 2013) and inhibition of carbon assimilation enzymes (Guo *et al*., 2005) chlorophyll reduction in *C. moschata* caused by MWMV infection in this study could be said to be due to a reduction in the number of chloroplast in the inoculated leaf tissues. Results of our study underline the fact that plant viruses unfavorably influence crops physiological processes.

Reduction in chlorophyll content led to a corresponding decrease in carbohydrate. Goodman *et al*. (2008) and Singh and Shuka (2009) also reported reduction in carbohydrate in other host plant virus combination. It has been established in many host virus infection that there is an increased demand of abnormal protein production required for the rapid synthesis of virus particle and for this purpose there has been increase in the diversion of assimilated carbon compounds towards protein synthesis eventually resulting in the decreased production of carbohydrate level in the leaves (Singh and Shuka, 2009). The decrease in the content of carbohydrate is due to increased respiration resulting from virus infection and the conversion of carbohydrate into amino acids used in protein synthesis of the viral coat. This reduction might also be due to the duration of virus infection (Muqit *et al*., 2007; Sinha and Srivastava, 2010).

CONCLUSION

From the results obtained it is clear that infection of *C. moschata* by a Nigerian strain of MWMV caused decreases in physiological and biochemical parameters. These reductions are of great concern both to the plant and to man who depend on this vegetable as a cheap source of mineral elements. Resources should be geared towards prevention of MWMV on *C. moschata* in order to ensure quality and guarantee-nutritive value by searching for resistant varieties of the vegetable and their cultivation are recommended.

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