EFFECTS OF MOROCCAN WATERMELON MOSAIC VIRUS (MWMV) ON THE GROWTH AND COMPOSITION OF ELEMENT AND ANTIOXIDANTS IN *Cucumeropsis edulis* NAUDIN

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ABSTRACT

Effects of *Moroccan Watermelon Mosaic* Virus (MWMV) on the growth and composition of elements and antioxidants in *Cucumeropsis edulis* were investigated using complete randomized designed experiment. Test plants were mechanically inoculated with MWMV on the 8th day. A set of un-inoculated plants served as the control. The growth of infected and control plants were evaluated after two months using linear measurements and biomass weight determination. The elemental and phytochemical properties of the plant extracts were determined by standard procedures. The results showed that MWMV caused significant reductions (P<0.05) in all the growth parameters of *Cucumeropsis edulis* with mean shoot height and dry leaf weight being 52.82 ± 6.15 cm and 0.40 ± 0.31 g in infected sample compared to the corresponding healthy values of 75.00 ± 13.24 cm and 1.23 ± 1.00 g recorded for the control. All the elemental contents of MWMV – infected plant were significantly (P<0.05) reduced with zinc having the least mean value of 0.08 ± 0.21 mg/100 g as against 1.83 ± 0.00 mg/100 g obtained from uninfected plants. Antioxidants such as alkaloid, flavonoid, saponin, tannin and terpene were detected but their presence varied between the infected and healthy plant samples. There is need to control the spread of this virus through phytosanitational measure, use of resistant variety and effective quarantine services to improve quality and ensure food security.

INTRODUCTION

In tropical Africa, *Cucumeropsis edulis* is grown for food and as a source of oil (Obute *et al.,* 2007). This plant can grow up to 5-10m long, climbing by simple tendrils with stem being angular and hairy. The fruit is egg-shaped, up to about 19cm long and 8 cm wide and cream in colour (Obute *et al.,* 2007). The farmer sows the seeds near dead trees at the edge of garden (Egunjobi and Adebisi, 2004). In Nigeria, it is grown in the rainforest belt between March – May (Owolabi *et al.,* 2012) where the demand for the seeds particularly in towns has led to large scale planting (Adewusi *et al.,* 2000). It is of great economic importance (Zoro Bi *et al.,* 2004). The seeds are used in preparing dough or sauce in Africa traditional societies as they constitute an important lipid and protein source (Ponka *et al.,* 2005). In Ghana, the juice from *C. edulis* fruit and leaf mixed with other ingredients is applied to the navel of a new born baby for five days till the cordrelic drops off (Burkill, 1985).

Plant viruses are responsible for a wide range of economic losses associated with crop production (Hull, 2002). Amongst the plant viruses, is the Moroccan Watermelon Mosaic Virus (MWMV) which belongs to the group Potyvirus (Family: *Potyviridae*). This virus is characterized by flexuous rods of about 730 nm in length. It was first reported in 1972 to cause severe diseases of various cucurbits in Morocco (Fischer and Lockhart, 1972). Growth and elemental alterations have been reported to occur in crops as a result of viral infection (Owolabi *et al.,* 2012). Altered phytochemicals in virus infected crops have been reported (Wood, 1990; Fallah *et al.,* 2009. Fischer and Lockhart (1972) have remarked that *Moroccan watermelon mosaic virus* (MWMV) could be a major threat to cucurbit production. Hence, the present study was undertaken to assess the effects of *Moroccan watermelon mosaic virus* (MWMV) on growth, some elemental and phytochemical contents of the leaf of *Cucumeropsis edulis.*

MATERIALS AND METHODS

Source of Seeds

Seeds of *Cucumeropsis edulis* used in this study were sourced from Itam Main Market in Itu Local Government Area of Akwa Ibom State.

Virus Source and Preparation of Inoculum

The virus designated as MWMV was isolated from *Coccinia barteri* Benth. The virus was propagated and maintained on *Cucumeropsis edulis* in the greenhouse of the Department of Botany, University of Calabar, Calabar. Virus inoculum was prepared according to the procedure of Thongmearkon *et al.* (1978) by grinding virus infected *Cucumeropsis edulis* leaves with buffer (0.05 M) potassium phosphate, pH 7.5 using sterile pestle and mortar.

Experimental Design and Inoculation of Experimental Plants

Seeds of *Cucumeropsis edulis* were planted between March and May 2016. The plants were arranged in twenty rows of five replicates using perforated polyethylene bags each filled with 4.2 kg of treated loamy soil. The experiment was laid out in a complete randomized design with ten rows of virus inoculated plants while another ten rows served as control. Prior to inoculation (8 days after planting), the surface of the leaves were dusted with carborundum power. Thereafter, the inoculum was applied by the conventional leaf-rub method.

Effect of MWMV on the Growth of Plants

The effect of MWMV on the growth of 60 day old test plants was evaluated. The shoot height was determined by measuring shoot height (cm) from the base to the tip of the plants. Measurements were taken for three replicates. The leaves of inoculated and healthy plants were counted visually from each plant. Averages of triplicate determinations were determined.

Leaves of the same age and position on infected and healthy plants were harvested and transferred to the laboratory where the leaves area was traced on graph and the total area calculated based on the number of squares within the traced region (Ting, 1982). The leaf length and width were carefully measured using a meter rule while the leaf fresh weight was measured with the aid Weighing Balance (DHG9053A, Ocean Med. England). Leaf dry weight was determined by drying leaf samples at temperature of $70^{O}C$ for 24 hours. Samples were dried and weighed three times using Blauscal Weighing Balance (DHG 9053A, Ocean Med. England). The harvested plants were placed in a bucket of water and the soil particles gently washed off. The shoots were cut off from the roots using scissors and then fresh weights determined and recorded. The samples were then oven-dried at $70^{O}C$ for 24 hours and the dry weight by weighing repeatedly to have a constant weight (Miyashi *et al.,* 1996).

Elemental Analysis of Plant Leaf

The infected and healthy leaf samples were oven-dried and reduced into powdery form. Digestion of the samples followed dry digestion method after which the minerals were determined using standard procedures as described by AOAC (2005).

Phytochemical Screening of Plant Leaf

Phyto-chemical tests were carried out on the aqueous powdered extracts using standard methods described by Banso (2009).

(a) Alkaloids

A 2.0 g of each extract was stirred with 5 ml of 1% aqueous hydrochloric acid on a steam bath. 1 ml of the filtrate was treated with a few drops of dragendorff's reagent. The formation of organ colour indicated the presence of alkaloids.

(b) Flavonoid
2g of each extract was heated with 10 ml of ethyl acetate over a steam bath for 3 mm. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow colouration was observed indicating a positive test for flavonoids.

(c) Saponins
In this test, 2.0 g of each extract was boiled in 20 ml of distilled water in a water bath and filtered. 10 ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent forth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

(d) Cyanogenic Glycosides
The plant extract (2.0 g) was added and mixed with 5 ml of chloroform. A few drop of conc. H_2SO_4 was added to the filtrate. A brown ring at interface or a violet ring may appear below the brown ring as positive test of cyanogenic glycosides.

(e) Tannins
The extract (0.5 g) was boiled in 20 ml of distilled water in a test tube and then filtered. A few drop of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration.

(f) Terpenes:
In this test, 2.0 g or each sample was mixed with 2 ml of $CHCl_3$ in a test tube. 3 ml of conc. H_2SO_4 was carefully added to the mixture to form a layer. An interface with a reddish brown colouration is formed if terpene is present.

Statistical Analysis
The Data obtained were analyzed using the student *t-test* by comparing the control with infected sample. Mean values were compared at 95% level of significant using statistical package for social science (SPSS) version 17.0.

RESULTS

The growth parameters of *Cucumeropsis edulis* infected with *Moroccan watermelon mosaic virus* (MWMV) are summarized in Table 1.

Table 1: Growth Parameters of *Cucumeropsis edulis* Infected with *Moroccan watermelon mosaic virus* (MWMV)

Growth Parameters	Infected Sample	Health Sample
Shoot height (cm)	52.82 ± 6.15*	75.00 ± 13.24
Leaf number	7.00 ± 0.31*	12.60 ± 1.43
Leaf length (cm)	5.00 ± 0.11*	9.60 ± 1.33
Leaf width (cm)	7.31 ± 0.12*	8.22 ± 0.34
Fresh shoot weight (g)	16.40 ± 1.24*	18.20 ± 2.47
Dry shoot weight (g)	1.40 ± 0.24*	1.80 ± 0.20
Fresh leaf weight (g)	7.40 ± 0.81*	10.96 ± 1.66
Dry leaf weight (g)	0.40 ± 0.31*	1.23 ± 1.00
Leaf area (cm^2)	12.90 ± 3.18*	67.33 ± 47.29

Values are means ± SEM, n = 3 replicates, P<0.05 * significant

The results revealed that MWMV caused significant reductions (P<0.05) in all the growth parameters of *Cucumeropsis edulis.* The least mean value of 0.40 ± 0.31 g was obtained for dry leaf weight in infected sample whereas the healthy sample had the corresponding mean value of 1.23 ± 1.00 g. The virus also reduced the mean shoot height in infected sample to 52.82 ± 6.15 cm whereas uninfected plant had 75.00 ± 13.24 cm. All the elemental contents of MWMV – infected plants were significantly (P<0.05) reduced (Table 2). Zinc had the lowest mean value of 0.08 ± 0.21 mg/100 g compared to 1.03 ± 0.00 mg/100 g recorded for healthy sample. The results of the qualitative phytochemical screening of the crude extracts of *C. edulis* revealed the presence of alkaloids, flavonoids, saponins, tannins and terpenes in varying concentrations in both infected and healthy samples (Table 3). Cyanogenic glycoside was not detected in the plant extracts.

Table 2: Mineral Contents of the Leaf of *Cucumeropsis edulis* Infected with *Moroccan watermelon mosaic virus* (MWMV)

Minerals	Infected Sample (mg/100g)	Health Sample (mg/100g)
Potassium	24.34 ± 0.10*	27.61 ± 0.11
Magnesium	11.21 ± 2.11*	14.02 ± 0.10
Copper	3.45 ± 0.00*	5.18 ± 0.03
Calcium	21.22 ± 2.30*	25.11 ± 2.10
Iron	6.70 ± 0.33*	9.80 ± 0.00
Na	12..33 ± 0.11*	15.30 ± 0.13
Potassium	7.60 ± 0.20*	9.61 ± 0.10
Phosphorus	13.83 ± 0.30*	25.08 ± 0.40
Zinc	0.08 ± 0.21*	1.03 ± 0.00

Values are means ± SEM, n = 3 replicates, P<0.05 *

Table 3: Qualitative Phytochemical Composition of the Leaf of *Cucumeropsis edulis* Infected with *Moroccan watermelon mosaic virus* (MWMV)

Chemical Constituent	Infected Sample	Healthy Sample
Alkaloids	++	+++
Flavonoids	+	++
Saponins	+	++
Cyanogenic glycosides	–	–
Tannins	++	+++
Terpenes	+	++

Key:
+ = Fairly present, ++ = Moderately present, +++ = Highly present, – = absent

DISCUSSION

This research presents the effects of *Moroccan watermelon mosaic virus* (MWMV) on growth, some elemental and phytochemical contents of the leaf of *Cucumeropsis edulis.* The research findings have shown that MWMV infection resulted in significant reduction in growth and elemental contents of *Cucumeropsis edulis.* This is in line with the work of Pawar *et al.* (1990) who reported reductions in shoot height, leaf weights as well as the leaf number of sorghum infected with *Sorghum ringspot virus* (SRSV). Similarly, El-Dougdoug *et al.* (2005) reported that potato virus reduced the number of leaves and heights of infected plant when compared with the growth of infected sample. Attack by pathogens including viruses usually alter plant metabolism leading to reduction in growth (Heil and Boston, 2005). Growth in plants is a complex phenomenon linked with numerous physiological processes (Owolabi *et al.,* 2012).

Elemental alterations observed in the present study are similar to the findings of Owolabi *et al.* (2012) who reported that infection of *Coccinia barteri* by MWMV caused reductions in the nutritional quality of the leafy vegetable as important dietary minerals such as magnesium, iron, calcium and vitamins A and C were significantly reduced. On the other hand, Frazer (1987) confirmed that the amounts of mineral elements in virus infected plants are usually altered.

Generally, plants make use of substantial quantities of mineral elements for their growth. They can be either macro-elements (required in relatively large amounts) or micro-elements (required only in very small amounts). Plants need potassium in large amount for photosynthesis and cambial activity whereas magnesium serves as a metabolic constituent of chlorophyll (Mehrotra and Aggarwal, 2006). The micronutrients such as iron, copper and zinc are required by plants in little amounts. Excessive supplies of these micronutrients have long been known to produce toxic effects on plants (Mehrotra and Aggarwal, 2006). In plant nutrition, magnesium serves as a component of chlorophyll. Deficiency of magnesium invariably results in extensive interveinal chlorosis of the leaves. Potassium acts as a component of nucleic acids, phospholipids and adenosine triphosphate (ATP). Potassium deficiency inhibits synthesis of protein while carbohydrate is checked (Verma, 2009). Iron is an electron carrier in the oxidation reduction in respiration and is a constituent of certain enzymes (Mehrotra and Aggarwal, 2006). Its deficiency seriously impairs aerobic respiration (Udoh *et al.,* 2005).

Calcium is perhaps the main constituent of the middle lamella. Deficiency symptoms are interveinal chlorosis and poor plant development (Udoh *et al.,* 2005). Cooper and manganese are trace elements required by plants in little amounts. Copper is involved in chlorophyll formation, ethylene synthesis and activity in fruit ripening (Verma, 2005). Lack of copper results in chlorosis. Manganese has a role in nitrogen metabolism and enzymes activation. Deficiency results in chlorosis of leaves and tissues as observed MWMV infected plants.

In this study, the reduction in the concentrations of alkaloid, flavonoid, saponin, tannin and terpene in infected *C. edulis* suggests a decrease in plant total fresh weight which is a common feature and important economic aspect of viral disease (Wood, 1990). These findings agree with altered phytochemicals in virus infected plants as reported by (Fallah *et al.,* 2005 and El-Dougdoug *et al.,* (2007). Phytochemicals are a large group of plant-derived compounds hypothesized to be responsible for much of disease protection (Arts and Hollman, 2005).

CONCLUSION AND RECOMMENDATIONS

In conclusion, MWMV-infection of *Cucumeropsis edulis* produced more significant ($P<0.05$) alterations on its growth, some mineral and phytochemical contents when compared with the uninfected plant. To ensure food security and poverty alleviation, the following recommendations are made: (i) Phytosanitation at all stages of plant propagation is needed, (ii) Planting of resistant varieties should be encouraged and (iii) More quarantine offices should be opened by government for certification of plant materials.

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