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Effects of *Moroccan Watermelon Mosaic Virus* (MWMV) on Growth, Some Elemental and Phytochemical Composition of the Leaf of *Cucumeropsis edulis* Naudin

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ABSTRACT

Effects of *Moroccan watermelon mosaic virus* (MWMV) on Growth, Some Elemental and Phytochemical Composition of the Leaf of *Cucumeropsis edulis* Naudin were carried out. Test plants arranged in complete randomized designed were mechanically inoculated at 8 days except the control. Two months post inoculation, infected and healthy plants were harvested and studied for growth parameters using graphical method, weighing and linear measurements. The elemental contents of both infected and uninfected plants were evaluated using standard methods proposed by Association of Official Analytical Chemists. Phytochemical tests were performed on the plant extracts following 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 obtained 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 while uninfected sample had 1.83 ± 0.00 mg/100 g. The phytochemical results of the crude extracts of *C. edulis* revealed the presence of alkaloid, flavonoid, saponin, tannin and terpene, all in varying concentrations in both 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. This will provide food security and poverty alleviation.

Keywords: *Cucumeropsis edulis,* Virus, Growth, Elemental and Phytochemical Composition.

INTRODUCTION

Cucumeropsis edulis Naudin is a species of melon belonging to the family *Cucurbitaceae.* The Yoruba, Hausa and Igbo people of Nigeria refer to the plant as "Egusi-itoo", "Agushi" and "Ebele" respectively. The Efiks and Ibibios of Cross River and Akwa Ibom States call it "Ikpan eyong". 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 wide and cream in colour (Obute *et al.,* 2007). The farmer sows the seeds near dead tress 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 to prepared dough or sauce in Africa traditional societies as they constitute an important lipid and protein sources (Ponka *et al.,* 2005). The juice from *C. edulis* fruit and leaf mixed with other ingredients is applied in Gahana 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) defined virus as "a set of one or more nucleic acid template molecules, normally encased in protein coat, which is able to organize its own replication only within suitable host cells". One of the plant viruses is the *Moroccan watermelon mosaic virus* (MWMV) which belongs to the genus potyvirus (Family: *Potyviridae*). This virus is characterized by flexuous rods of about 730 nm in length. It was first reported in 1972 causing 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 (Heil and Boston, 2002; Owolabi *et al.,* 2012). Altered phytochemicals in virus infected crops have been reported (Wood, 1990; Fallah *et al.,* 2009 and Srivastava, 2010). 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 by Dr. A. T. Owolabi of the Department of Botany, Faculty of Science, University of Calabar, Nigeria. 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:

The planting of seeds of *Cucumeropsis edulis* was carried out 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 the Virus on Shoot Height and Number of Leaves:

Sixty days post-inoculation, the effects of the virus on 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 considered.

Effect of the Virus on Leaf Area:

Leaves of the same age and position on infected and healthy plants were harvested in polyethylene bags and brought to Biology Laboratory of the

Department of Science Technology, Akwa Ibom State Polytechnic, Ikot Osurua. The area was traced on the graph and total area calculated based on the number of squares within the traced region (Ting, 1982).

Effect of the Virus on Leaf Length and Width:

Infected and healthy leaves were placed on a clean specimen board before their lengths and widths were carefully measured using metre rule. Average of triplicate measurements were taken for each plant.

Effect of the Virus on Fresh and Dry Weights of Leaves:

Leaf fresh weight was taken for each sample by measuring using 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).

Effect of Virus on Fresh and Dry Weights of Shoots:

The harvested plants were placed in a bucket of water and the soil particles gently washed off. The shoots were cut off from the roots using scissors and then fresh weights determined and recorded. The samples were later oven-dried at 70^{O}C for 24 hours. They were then weighed three times for both infected and healthy samples (Miyashi *et al.,* 1996).

Mineral Analysis

The mineral analyses were conducted in the Department of Biochemistry, University of Uyo, Uyo. 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

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

Test for 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.

Test for Flavonoid:

A 2.0 g 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.

Test for Saponins:

A 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.

Test for Cyanogenic Glycosides:

The plant extract (2.0 g) was added and mixed with 5 ml of chloroform. A few drops 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.

Test for Tannins:

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

Test for Terpenes:

A 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. 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 respectively. 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 obtained for the 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 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

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.* From this study, MWMV resulted in significant reductions of all the growth parameters 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.* (2010) 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. 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

In conclusion, MWMV-infection of *Cucumeropsis edulis* produced more significant ($P<0.05$) alterations on its growth, some mineral and phytochemical contents than as obtained for the uninfected plant.

RECOMMENDATIONS

To secure food security and poverty alleviation, the following recommendations have become necessary:

1. Phytosanitation at all stages of plant propagation is needed.
2. Planting of resistant varieties should be encouraged.
3. More quarantine offices should be opened by government for certification of plant materials.

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