

THE PATHOGENICITY OF CUCUMBER MOSAIC VIRUS (CMV) AND PUTATIVE BITTERLEAF MOSAIC VIRUS (BLMV) ON *Celosia argentea*

*Aliyu, T.H., Balogun, O.S., Osumah, R., and Adeoti, O.M.

Department of Crop Protection, Faculty of Agriculture, University of Ilorin.
P.M.B 1515, Ilorin - Kwara State Nigeria.

E-mail of corresponding author: aliyutaiyehusseini@yahoo.com

Phone: +2348030472667

Abstract

A greenhouse study was conducted to determine the pathogenic effect of *Cucumber mosaic virus* (CMV) and Putative *Bitterleaf mosaic virus* (BLMV) on a local variety of *Celosia argentea*. The viral inoculi were extracted by homogenization from infected leaves of cowpea and bitterleaf that were obtained from the International Institute of Tropical Agriculture (IITA) Ibadan. Seeds of *Celosia argentea* were sown in plastic pots that were filled with sandy loam soil, previously steam sterilized at 120°C for 2 hours. The plants were mechanically inoculated 3 weeks after planting with either CMV, BLMV or Buffer (control). Each treatment was replicated 4 times in a Completely Randomized Design (CRD) manner. The results of the experiment revealed that the *C. argentea* was susceptible to infection with CMV and BLMV. Percentage severity of the viral infections were significantly different ($p=0.05$) compared with the control. The results indicated that CMV had the significantly highest effect on percentage disease severity (53.1%), followed by BLMV (16.5%) and control plants had no infection. Subsequent growth and yield attributes in the virus inoculated plants were also generally lower than those recorded for the control. The significantly lowest stem girths were recorded in plants inoculated with CMV (1.6cm), followed by BLMV (2.8cm), while the significantly highest stem girths were obtained in the control plants (4.2cm). The study found *C. argentea* to be susceptible to infection by CMV and BLMV and thus advocates the need for effective control measures for high productivity of the vegetable.

Key Words: *Celosia argentea*, Pathogenicity, Cucumber Mosaic Virus, Bitterleaf mosaic virus, Inoculation.

Introduction

Celosia argentea belongs to the family *Amaranthaceae* and is one of the important leafy vegetable commonly found in traditional intercropping system of the tropics. It is commonly known as ‘Sokoyokoto’ or ‘Ajefawo’ amongst the Yorubas (Schippers, 2000) and is a vegetable of high economic value for most rural vegetable farmers (Akinfasoye *et al.*, 2008).

The leaves have also been found to suppress elevation of post prandial blood glucose levels in humans and are rich sources of vitamin A and C (Innami *et al.*, 2005). The seeds also possess broad antibacterial properties (Pall *et al.*, 2006). In experiments done in Nigeria, *Celosia argentea* was found to suppress weeds more than weeding twice when inter-planted into chewing cane (Busari *et al.*, 1997).

Celosia argentea is susceptible to pathogens which cause fungal, nematode, bacterial, and viral diseases (Broadbent, *et al.*, 1977., Caveness and Wilson, 1977., Nyamupingidza and Machakaire, 2003). The productivity of this vegetable is therefore largely dependent on the type of disease control strategy employed during cultivation (Siddiqui and Alam, 1989).

Viruses constitute a major limiting factor to vegetable production in most African countries (Nono-Womdim, 2003). *Amaranthus mosaic virus* (AMV) was reported for the first time in Nigeria by Taiwo *et al.* (1988), and since then AMV has been found to be highly prevalent in Lagos with an incidence rate of 19.7% (Taiwo and Owolabi, 2004).

Cucumber mosaic virus (CMV) has one of the broadest host ranges. CMV as a type species of the genus *Cucumovirus* in the family *Bromoviridae*, and is reported to infect 1287 plant species in 518 genera belonging to 100 families (Edwardson, Christie, 1987). The most common symptom induced by CMV is mosaic; however, severity of the disease may range from no obvious symptoms in some crops to death of the host species (Dijkstra and Khan, 2006). Tospoviruses on the other hand are rated among the ten most devastating plant viruses (Peters *et al.*, 1991). The impact of tospoviruses is enormous, mainly due to their extremely broad host range, which exceeds 650 different plant species in more than 70 distinct botanical families and the host range include bitter leaf (Goldbach and Peters, 1994).

Celosia argentea constitute a major part of the peoples’ diet in the middle belt and southern parts of the country where they are

mostly used in soups. Small scale farmers also derive income from production of the crop during dry seasons in different parts of Nigeria. Virus diseases have been recognized to constitute one of the major factors limiting vegetable crop production worldwide (Grogan, 1980). It is therefore important to determine the pathogenicity of some endemic viruses on *C. argentea* to enable the deployment of effective control measures.

The Objective therefore was to evaluate in greenhouse studies, the pathogenicity of two viruses, *Cucumber mosaic virus* (CMV) and *Bitterleaf mosaic virus* (BLMV) on *Celosia argentea*

Materials and Methods

Experimental design and agronomic practices

The study was conducted in the greenhouse of the Crop Protection Department-Faculty of Agriculture, University of Ilorin. It is located in the Southern Guinea savanna agroecology of Nigeria on latitude 8° 26' N, longitude 4° 29' E, and about 344.7m above sea level. The seeds of *Celosia argentea* were obtained from the Ipata Market in Ilorin. Four seeds (later thinned to two stands at 7days after planting) were sown in each plastic pot that were filled with sandy loam soil that was previously steam sterilized at 120°C for 2hours. Urea fertilizer was applied prior sowing at the rate of 20 Kg/ha.

Source of inoculi and inoculation procedure

The viral isolates (CMV and BLMV) were sourced from the International Institute of Tropical Agriculture (IITA) Ibadan Oyo State - Nigeria. The inoculi were prepared by grinding systematically leaves of crops infected by the viruses (i.e. cowpea leaves and bitter leaf leaves), separately in 0.01M phosphate buffer at pH 7.0 using sterile mortar and pestle. Leaf-to-buffer ratio was 1:5 (1g of infected leaf to 5ml buffer). Inoculation was carried out 21days after planting on the first and second leaves from the stem base of *Celosia* plants. The plants were lightly dusted with carborundum and rubbed with cotton wool soaked in the plant sap. Control plants were mock-inoculated with buffer only. Leaves were later drained with sterile water for adequate light interception (Balogun, 2000). All the plants were then kept in the greenhouse at a temperature of 27–32°C.

Experimental Design, data collection and analysis

The three treatments were: (i) *C. argentea* inoculated with *Cowpea mosaic virus* (CMV) (ii) *C. argentea* inoculated with *Bitterleaf mosaic virus* (BLMV) (iii) *C. argentea* inoculated with buffer (Control). Each treatment was replicated 4 times giving a total of 12 treatments/observation units and the experiment was laid out as a Completely Randomized Design (CRD). Data were collected on weekly basis from the 1 week after inoculation on the following parameters: (a) Number of leaves per plant: this was determined by counting all the leaves on each plant. (b) Number of leaves per plant manifesting mosaic symptoms (c) The percentage disease severity: this was calculated by the number of diseased leaves relative to the total number of leaves on any given plant and expressed as percentage and according to the method of Aliyu *et al.*,(2012). (d) Plant height: this was done using a meter rule. (e) Stem girth at harvest: this was measured using a white thread and a ruler and according to the method of Aikins *et al.*,(2012).

All data collected were thus subjected to appropriate statistical analysis and the mean values were partitioned using the New Duncan Multiple Range Test and the Least Significant Difference at 5% level of significance.

Results

Effect of viral inoculation on disease severity

Table 1 shows the effect of the viral inoculations on the percentage severity of the disease on the *Celosia argentea*. The result showed that percentage severity of the viral infection were significantly different ($p=0.05$) compared with the control. The inoculation with CMV had the significantly highest effect on percentage disease severity (53.1%), followed by BLMV (16.5%).The control plants were not infected.

Table 1: Effect of viral inoculation on percentage severity of the viral infection

Treatment	Severity (%)
Bitterleaf mosaic virus	16.5 ^b
Cucumber mosaic virus	53.1 ^a
CONTROL	0 ^c
S.E.M (+/-)	0.029

In each column, means followed by the same letter are not significantly different at $p=0.05$ according to Duncan's Multiple Range Test.

Plate 1 shows the virus inoculated celosia plants in comparison to the control plants (Plate 2) at 14DPI. The severity of the viral

inoculations is manifested by mosaic and mottling symptoms. The control plants



Plate 1: Mosaic/Mottling on *C. argentea* at 14DPI



Plate 2: Healthy *C. argentea* at 14DPI

Effect of viral inoculation on plant height

The analytical results presented in Table 2 shows that the highest reductions in plant height resulted from the inoculations with CMV and BLMV. The table indicates that from the 14th to the 28th day post inoculation (DPI), the significantly shortest plants were those that were inoculated with the viral pathogens. By the 35th DPI however, the plants that were inoculated with CMV were the most stunted with an average height of 21.1cm, followed by the plants inoculated with BLMV (22.4cm). The control however produced the significantly tallest plants (24.1cm).

Table 2: Effect of viral inoculation on plant height (cm)

Treatment	14DPI	21DPI	28DPI	35DPI
Bitterleaf mosaic virus	7.4 ^b	15.2 ^b	17.3 ^b	22.4 ^b
Cucumber mosaic virus	7.8 ^b	14.7 ^b	16.3 ^b	21.1 ^b
CONTROL	9.8 ^a	17.1 ^a	18.7 ^a	24.1 ^a
S.E.M (+/-)	0.410	0.951	0.966	1.317

DPI = Days Post Inoculation. Each value is the mean of four replicates. In each column, means followed by the same letter are not significantly different at $p=0.05$ according to Duncan's Multiple Range Test

Effect of viral inoculation on mean number of leaves per plant

Table 3 reveals the effect of viral inoculation on the mean number of leaves per plant. It indicates that the viral inoculations of BLMV and CMV caused significant reductions and fewer production of leaves per plant compared to the control. It shows that at 14DPI, the CMV inoculated plants were the most severely affected and the significantly

however looked apparently healthy (Plate 2).

lowest mean number of leaves per plant (7.2). This trend was continuous to the 42DPI.

Table 3: Effect of viral inoculation on number of leaves per plant

Treatment	14DPI	21DPI	28DPI	35DPI	42DPI
Bitterleaf mosaic virus	7.8 ^b	9.4 ^{ab}	13.4 ^{bc}	15.3 ^b	17.0 ^b
Cucumber mosaic virus	7.2 ^b	8.6 ^b	11.2 ^c	11.5 ^c	11.8 ^c
CONTROL	8.1 ^a	10.5 ^a	14.2 ^a	17.2 ^a	22.1 ^a
S.E.M (+/-)	0.410	0.393	0.822	0.868	1.066

DPI = Days Post Inoculation. Each value is the mean of four replicates. In each column, means followed by the same letter are not significantly different at $p=0.05$ according to Duncan's Multiple Range Test.

Effect of viral inoculation on stem girth at harvest

Table 4 shows that irrespective of viral inoculum, the pathogens caused a significant reduction in the stem girth at harvest of the celosia plants. The significantly lowest stem girth values were however recorded for plants inoculated with CMV (1.6cm), followed by BLMV (2.8cm). The stem girths were significantly highest in the control plants (4.2cm).

Table 4: Effect of viral inoculation on stem girth (cm) at Harvest

Treatment	42DPI
Bitterleaf mosaic virus	2.8 ^a
Cucumber mosaic virus	1.6 ^a
CONTROL	4.2 ^b
S.E.M (+/-)	0.671

Means followed by the same letter are not significantly different at $p=0.05$ according to Duncan's Multiple Range Test

Discussion

The results from the study showed that the two viruses inoculated resulted in the development of mosaic/mottling symptoms which negatively affected growth parameters of *C. argentea*. It further showed that most of

the leaves produced after inoculation remained small with a yellowish-green appearance. Plant viruses cause symptomatic phenotypes of disease pathological phenotypes as the result of interference and/or competition for a substantial amount of host resources, which can disrupt host physiology to cause disease. This interference/competition affects a number of genes, which seems to be greater the more severe the symptoms that they cause (Pallas and Garcí'a, 2011).

The effect of inoculation of the virus pathogens on disease severity indicated that the it caused symptomatic phenotypes of disease triggering pathological manifestations with fatal effect on the host plant. In similar experiments with virus pathogen, Komatsu *et al.* (2010) have shown that systemic necrosis in *Nicotiana benthamiana*, induced by *Plantago asiatica* mosaic virus infection, was associated with programmed cell death, biochemical features and gene expression patterns that are characteristic of hypersensitive reaction. The viruses in this study thus have positive impact on disease severity.

The virus inoculated plants were stunted and had reduced stem girths compared with the controls. Observations similar to these were made by Agrios and Walker (1985), while working on pepper plants mechanically infected with *Cucumber mosaic virus* (CMV) and it was attributed to virus infection.

Some symptoms of viral infections have been shown by Kasschau *et al.* (2003), to be as the result of alterations to plant growth and development. It can also be deduced from the present study that the changes observed in the physiology and morphology of the *C. argentea* associated is due to the CMV and BLMV infections. This is supported by Agrios (2005), that the effect of plant pathogens leads to the development of different symptoms which cause reduction in yield and quality of products.

The growth parameters such as number of leaves, height and stem girth were significantly decreased in CMV and BLMV infected *C. argentea* compared with the control plants. These results are in agreement with the observation made by Owolabi and Taiwo, (2001) while working on the effect of *Celosia mosaic virus* on *Celosia argentea* L. plants.

These growth parameters are directly related with photosynthetic activities which provide energy for growth. Therefore, a significant reduction in these parameters would cause a decline in overall growth and invariably a high percentage severity of infection as observed in the inoculated plants. These assertions were also propounded in similar findings by Wintermantel (2005) and by Taiwo and Akinjogunla, (2006).

Conclusion

This study shows that *C. argentea* is highly susceptible to infection by *Cucumber mosaic virus* (CMV) and *Bitterleaf mosaic virus* (BLMV) with the attendant reduction in growth and yield. There is the need therefore, for control measures against production losses resulting therein. Furthermore, laboratory and field studies are necessary for the understanding of transmission mechanism.

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