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PLANT AGE IN RELATION TO CUCUMBER MOSAIC VIRUS DISEASE SEVERITY ON OKRA (*Abelmoschus esculentus* L. Moench) IN SOUTHERN GUINEA SAVANNA AGROECOLOGY OF NIGERIA

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ABSTRACT

The severity of *Cucumbermosaicvirus*(CMV; genus *Cucumovirus*; family *Bromoviridae*) infection on okra (*Abelmoschusesculentus*) plants infected at different growth stages was investigated in Minna (6.44°E, 9.51°N; 220 m above sea level), Southern Guinea Savanna of Nigeria. Two field experiments were conducted simultaneously using Randomised Complete Block Design (RCBD) with four replications. Seeds of the okra cultivar "Ikeregi" which is commonly grown in Minna were sown in the third week of May, 2014 on 5-m long ridges at inter- and intra-row spacing of 75 cm × 30 cm. Treatments consisted of uninoculated plants (control), CMV-inoculated at 7 days after sowing (DAS), 14 DAS and 21 DAS. Plants were assessed for disease incidence, disease severity, growth and yield parameters. Data were subjected to analysis of variance (ANOVA). Significance of the *F*-test was determined at5 % probability level and means were separated using the Least Significant Difference (LSD). One hundred percent CMV infection was observed on all the plants regardless of time of inoculation. However, disease severity varied with time of infection. At 8 weeks after inoculation (WAI), disease severity was significantly ($p<0.05$) highest in the plants inoculated at 7 DAS (mean symptom score = 4.0), followed by those infected at 14 DAS (mean symptom score = 2.0), whereas the lowest value was observed in those infected at 21 DAS (mean symptom score = 1.0). Number of leaves per plant was significantly ($p<0.05$) highest in uninoculated plants (22 leaves per plant), whereas infection at 7 DAS resulted in the lowest number of leaves per plant (12 leaves/plant). The plants infected at 14 DAS had a mean of 16 leaves per plant while those inoculated with CMV at 21 DAS produced a mean of 21 leaves per plant, which was not significantly($p>0.05$) different from the mean observed in uninoculated plants.The fruit weight per plant of uninoculated plants (58.5 g) was not significantly different from those infected at 21 DAS (57.5 g). Conversely, infection at 7 DAS and 14 DAS resulted in fruit weight of 23.5 and 36.5 g, respectively. The present study showed that protecting plants for the first 21 DAS could significantly reduce CMV pathogenicity in okra fields. Therefore, okra farmers should implement adequate measures so as to reduce the effect of viruses infecting okra at early stages of growth. However, integrated pest management strategy such as cultivation of tolerant varieties and use of close spacing could be incorporated as control strategies.

Keywords: *Abelmoschusesculentus*, disease severity, pathogenicity, yield

INTRODUCTION

Okra (*Abelmoschusesculentus* L. Moench) is a member of the family Malvaceae and its origin has been traced to Asia and Africa (Kumar *et al.*, 2010). It is one of the economically important vegetable crops in tropical and sub-tropical parts of the world. Its immature fruits and leaves are consumed as vegetables or used in salad, soups and stews. Additionally, okra mucilage is used as a plasma replacement or blood volume expander (Gemede *et al.*, 2015). Okra seeds are valuable for oil and linoleic acid production (Ndunguru and Rajabu, 2004). Its fruits also contain carbohydrates and vitamins and play a vital role in human diet (Dilruba *et al.*, 2009). There is an increasing demand for vegetable oils derivable from okra seeds due to the growing human population and the expanding oil industry with health promoting oil components. At present, okra is cultivated as a vegetable crop but it has potential for cultivation as an essential oilseed crop because its seeds contain high amount of oil (20-40 %) (Sorapong, 2012). In Nigeria, okra cultivation is a dependable source of livelihood and income for many farmers (Asadu *et al.*, 2016).

Worldwide, about six million tonnes of okra is produced annually (Ngbede *et al.*, 2014). In Nigeria, okra is produced in all the agro-ecologies both in rainy and dry seasons (Bamire and Oke, 2003). It is cultivated in the country either as a sole crop or in mixed cropping system probably owing to its high adaptability to different soil types and wide acceptability for various purposes (Konyeha and Alatise, 2013). In spite of its numerous benefits, okra yield is seriously constrained by abiotic and biotic factors. Some of the major viral diseases of okra include those induced by *Okra leaf curl virus* (OKLCV) (Gamal and Ghanem, 2003), *Okra mosaic virus* (OkMV) (Ndunguru and Rajabu, 2004), and *Okra yellow vein mosaic virus* (OYVMV) (Ali *et al.*, 2012).

Cucumber mosaic virus induces a wide range of symptoms, depending on genetic background of the host, age of the plant at infection and virus strain (Alegbejo, 2015). Studies have shown that severity of infection is most conspicuous with attendant high loss when plants are infected before bloom (Zitter and Murphy, 2009). In highly susceptible cultivars, early infected plants may not produce pods because CMV causes flower abortion and abnormal development. The pods of severely infected plants are mostly curved, mottled and reduced in size. However, plants may recover and resume normal growth with limited yield loss in late infected plants (Zitter and Murphy, 2009). The virus is transmitted by aphids (Palukaitiś *et al.*, 2002). Mechanical transmission has also been reported. It is transmitted by more than 60 species of aphid, notably (*Aphis gossypii* Glover) and (*Myzus persicae* Sulzer) in a non-persistent manner. *Cucumber mosaic virus* can be acquired by aphids in 5-10 seconds and transmitted in less than 1 minute. However, transmission efficiency declines after about 2 minutes and is usually lost within 2 hours (Alegbejo, 2015).

Cucumber mosaic virus has been identified as a threat to several crops including vegetables. However, there is scarcity of information on its severity in okra fields. Virus infection can cause complete yield loss if appropriate control measures are not implemented. Therefore, this study was conducted to determine the severity of *Cucumbermosaicvirus* disease on okra plants infected at different growth stages.

MATERIALS AND METHODS

Study Location

Two experiments were conducted at the Teaching and Research Farm of the Department of Crop Production, Federal University of Technology, Minna (6.44°E, 9.51°N; 220 m above sea level) during the 2014 cropping season. Minna is located in the Southern Guinea Savanna agro-ecological zone of Nigeria with annual mean rainfall of 1200 mm. The raining season normally begins in April, peaks in September and ends in the first week of October. Temperatures fluctuate between 35 and 37.5 ^{o}C with relative humidity between 60 and 80 % in July and 40 to 60 % in January. The soil of the site originated from basement complex rocks and has been classified as Alfisol (Adeboye *et al.*, 2011).

Collection of Seeds

The most commonly grown okra cultivar "Ikeregi" in Minna was used for the study. Thirty okra pods were randomly selected from okra farm and seeds from each pod were ground in inoculation buffer at the rate of 1 g/mL. Extract of each okra sample was rubbed on 10-day old CMV-susceptible cowpea (TVU 76) seedlings to ascertain that the seeds were virus-free.

Serological Testing

Leaves from the inoculated cowpea seedlings were harvested at 3 WAI and tested for CMV using Enzyme-Linked Immunosorbent Assay (ELISA). The harvested leaves were ground in coating buffer at the rate of 1 g/mL using cold sterilized mortars and pestles (Kumar, 2009). One hundred microlitres of the leaf extract were loaded into duplicate wells of the microtitre plate (Thermo Scientific "Nunc", Milford, MA). Extract of the leaves from healthy cowpea seedlings was used as negative control. The plate was incubated at 37 ^{o}C for 1 h, washed three times with phosphate buffered saline-Tween (PBS-T) and tap-dried on a paper towel. Two hundred microlitres of a blocking solution were added to each well and the plate was incubated again for 30 min at 37 ^{o}C. One hundred microlitres of the CMV polyclonal antibody diluted (1:10, 000; v/v) with conjugate buffer were added to each well.

The plate was incubated at 37 ^{o}C for 1 h, washed thrice and 100 µL of the goat anti-rabbit antibody diluted with conjugate buffer (1:15,000) were added to the wells. The plate was incubated at 37 ^{o}C for 1 h, washed and 100 µL of *p*-nitrophenyl phosphate dissolved in substrate buffer was added to the wells. The plate was incubated at room temperature (37 ^{o}C) in dark and absorbance values were read at 405 nm (A_{405}) after 1 h, using a microplate reader (MRX, Dynex Technologies, Inc., USA). Absorbance readings were accepted to be positive when the mean of the duplicate wells exceeded two times the negative control (Kumar, 2009).

Crop Establishment and Inoculations

The experiments were laid out in a randomised complete block with four replications. Treatments consisted of uninoculated plants (control), CMV-inoculated at 7 days after sowing (DAS), 14 DAS and 21 DAS. Seeds of the okra cultivar "Ikeregi" which is commonly grown in Minna were sown in

the third week of May, 2014 on 5-m long ridges at inter- and intra-row spacing of 75 × 30 cm. The inoculum used was a severe Nigerian CMV isolate obtained from the stock in the Department of Crop production, Federal University of Technology, Minna. The physical and chemical properties of the isolate have been reported (Taiwo, 2001). The isolate was maintained on silica gel at 37 ^{o}C till used. CMV isolate was ground in inoculation buffer at the rate of 1 g/mL. At 10 days after sowing, carborundum powder (600-mesh) was dusted on the upper leaf surface and virus extract was rubbed on it. At 8 weeks after inoculation, the topmost leaves of the inoculated plants were collected and tested for CMV (Kumar, 2009).

Data Collection and Statistical Analysis

Disease incidence was taken as percentage of inoculated plants exhibiting CMV disease symptoms at 1 and 2 WAI. Disease severity was recorded at 2, 4, 6 and 8 WAI based on a visual scale (Arif and Hassan, 2002). In the scale:

1 = no symptoms (apparently healthy plant);
2 = slightly mosaic leaves (10-30 %);
3 = mosaic (31-50 %) and leaf distortion;
4 = severe mosaic (51-70 %), leaf distortion and stunting;
5 = severe mosaic (>70 %), stunting and death of plants.

Number of leaves per plant (2, 4, 6, and 8 WAI), plant height (2, 4, 6, and 8 WAI), number of fruits per plant, fruit diameter (8 WAI), fruit length (8 WAI) and fruit weight per plant were also recorded. Data were subjected to analysis of variance (ANOVA) and significance was determined at 5 % probability level. Means were separated using the Least Significant Difference (LSD). Statistical analysis was performed with statistical analysis system (SAS, 2008).

RESULTS

Effect of Plant Age at Infection on CMV Disease Incidence

One hundred percent disease incidence was observed in all the inoculated plants. Leaf mottling was first sighted in the plots inoculated at 7 and 14 DAS, at 1 WAI. In the plants infected at 21 DAS, symptoms of infection manifested at 13 days after inoculation. In contrast, uninoculated plants were apparently symptomless.

Effect of Plant Age at Infection on CMV Disease Severity

At 2 WAI, disease severity was significantly (p<0.05) highest (2.0) in the plants inoculated at 7 DAS. (Fig. 1). The lowest mean symptom score was observed in the plants infected at 21 DAS (symptom score =1.0) while a mean symptom score of 1.5 was observed in the plants inoculated at 14 DAS.At 4 WAI, the mean symptom score rose to 3.3 in the plants inoculated at 7 DAS. In the plants infected at 14 DAS the mean disease severity was 2.0, which was not significantly (p>0.05) different from 1.5 observed in the plants infected at 21 DAS.

At 6 and 8 WAI, the mean symptom score decreased to 1.0 in the plants infected at 21 DAS. The plants inoculated at 7 and 14 DAS showed a constant mean symptom score of 4.0 and 2.0,

respectively. In contrast, there were no visible symptoms of infection on uninoculated plants (score = 1). Virus concentration was significantly ($p<0.05$) highest in the plants infected at 7 DAS (absorbance = 1.5), followed by those inoculated at 14 DAS (absorbance = 0.8), whereas the lowest value was obtained in those infected at 21DAS (absorbance = 0.4) (Fig. 2).

Effect of Plant Age at Infection on Growth and Yield

At 2 WAI, number of leaves per plant was significantly ($p<0.05$) highest in uninoculated plants (13 leaves/plant), followed by those infected at 21 DAS (11 leaves/plant). Similarly, significantly ($p<0.05$) higher number of leaves was found in those inoculated at 14 DAS (8 leaves/plant) than those infected at 7 DAS (4 leaves/plant) plants. At 4 WAI, uninoculated plants produced a mean of 18 plants per plant which was not significantly ($p>0.05$) different from 15 leaves per plant encountered in those infected at 21 DAS. The number of leaves per plant (11 leaves) observed in the plants inoculated at 14 DAS was in turn significantly higher than those infected at 7 DAS (8 leaves). Although at 6 and 8 WAI, number of leaves per plant was highest in uninoculated plants (21 and 22 leaves/plant, respectively), it was not significantly ($p>0.05$) different from those infected at 21 DAS (20 and 21 leaves/plant, respectively). Infection at 7 DAS resulted in the lowest number of leaves per plant (9 and 12 leaves/plant, respectively). In the plants infected at 14 DAS, number of leaves averaged 14 and 16 per plant, at 6 and 8 WAI, respectively (Fig. 3).

Cucumber mosaic virus disease significantly ($p<0.05$) reduced plant height. Moreover, time of infection influenced the severity of CMV on plant height and the data on this parameter followed a trend similar to that of number of leaves per plant. Some of the plants inoculated at 7 DAS were stunted and exhibited poor growth. Uninoculated plants were consistently the tallest throughout the period of assessment (Fig. 4). At 2 WAI, the highest mean height was observed in uninoculated plants (21.5 cm), followed by those infected at 21 DAS (18.0 cm). The mean height (16.5 cm) of the plants inoculated at 14 DAS was also significantly ($p<0.05$) higher than those infected at 7 DAS (13.5 cm). From 4 to 8 WAI, changes in plant height followed a similar trend. At 8 WAI, reduction in plant height was 8.9 % in the plants inoculated at 21 DAS relative to uninoculated plants, and 58.5 % in those infected at 7 DAS while 40.5 % height reduction was observed in the plants inoculated at 14 DAS.

Number of fruits per plant was significantly ($p<0.05$) highest in uninoculated plants (21 fruits) and those infected at 21 DAS (21 fruits). Conversely, the lowest (8 fruits) number of fruit per plant was found in the plants inoculated at 7 DAS, resulting in 61.9 % reduction compared with uninoculated plants. In the plants infected at 14 DAS number of fruit per plant (15 fruits) was reduced by 28.6 % (Table 1). The fruit diameter of uninoculated plants and those inoculated at 21 DAS was similar (2. cm). On the other hand, fruit diameter was 0.4 and 1.0 cm in those inoculated at 7 and 14 DAS, respectively. Variation in fruit length followed the same pattern as recorded for fruit diameter (Table 1). Fruit length varied between 5.5 and 10 cm. The longest fruit was obtained from uninoculated plants (10 cm) and those infected at 21 DAS (10 cm)while those infected at 7 DAS exhibited the lowest value (5.5 cm). A mean fruit length of 8.5 cm was observed in the plants infected at 14 DAS.

The fruit weight per plant of uninoculated plants was the heaviest (58.5 g) but not significantly ($p>0.05$) different from those infected at 21 DAS (57.5 g). The lowest fruit weight (23.5 g) was found in the plants infected at 7 DAS which accounted for 59.8 % reduction in fruit weight. In contrast, the mean fruit weight of the plants infected at 14 DAS was 36.5 g, which accounted for 37.6 % fruit weight reduction (Table 1)..

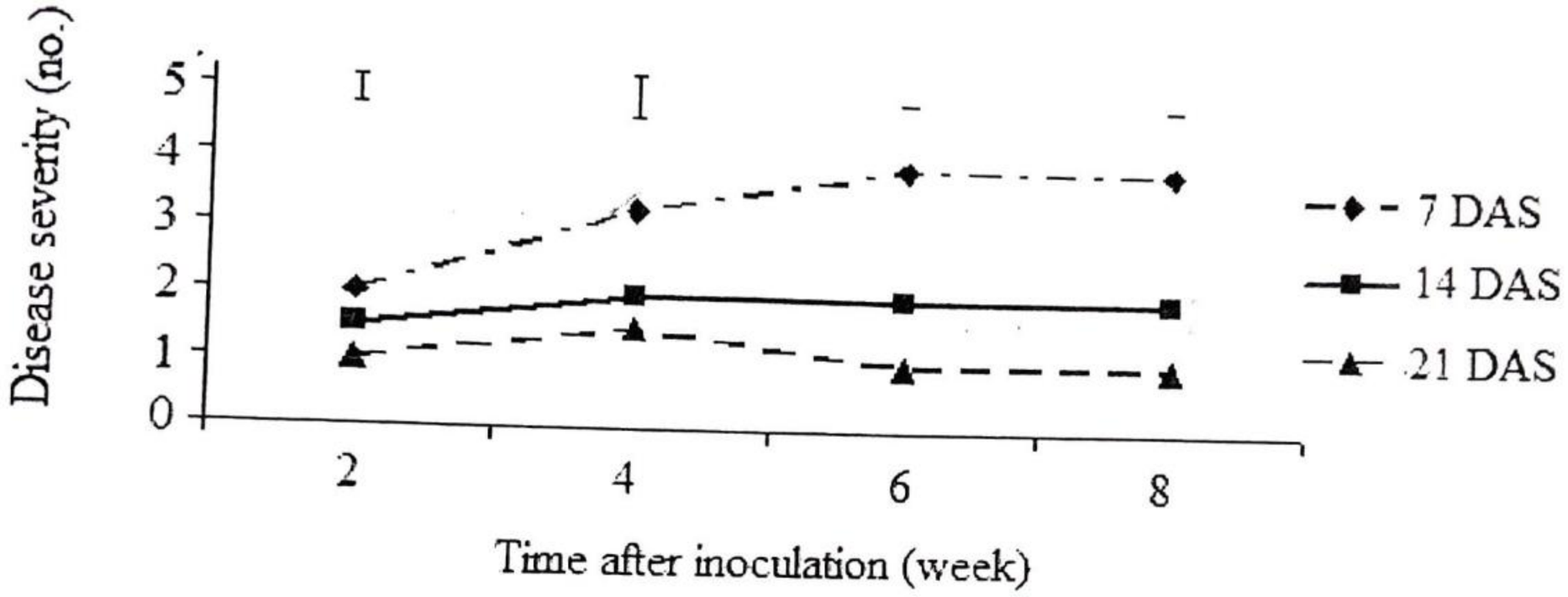

Fig. 1: Disease progress in okra plants inoculated with *Cucumber mosaic virus* at different days after sowing (DAS) during the 2014 cropping season in Minna
Vertical bars represent LSD values

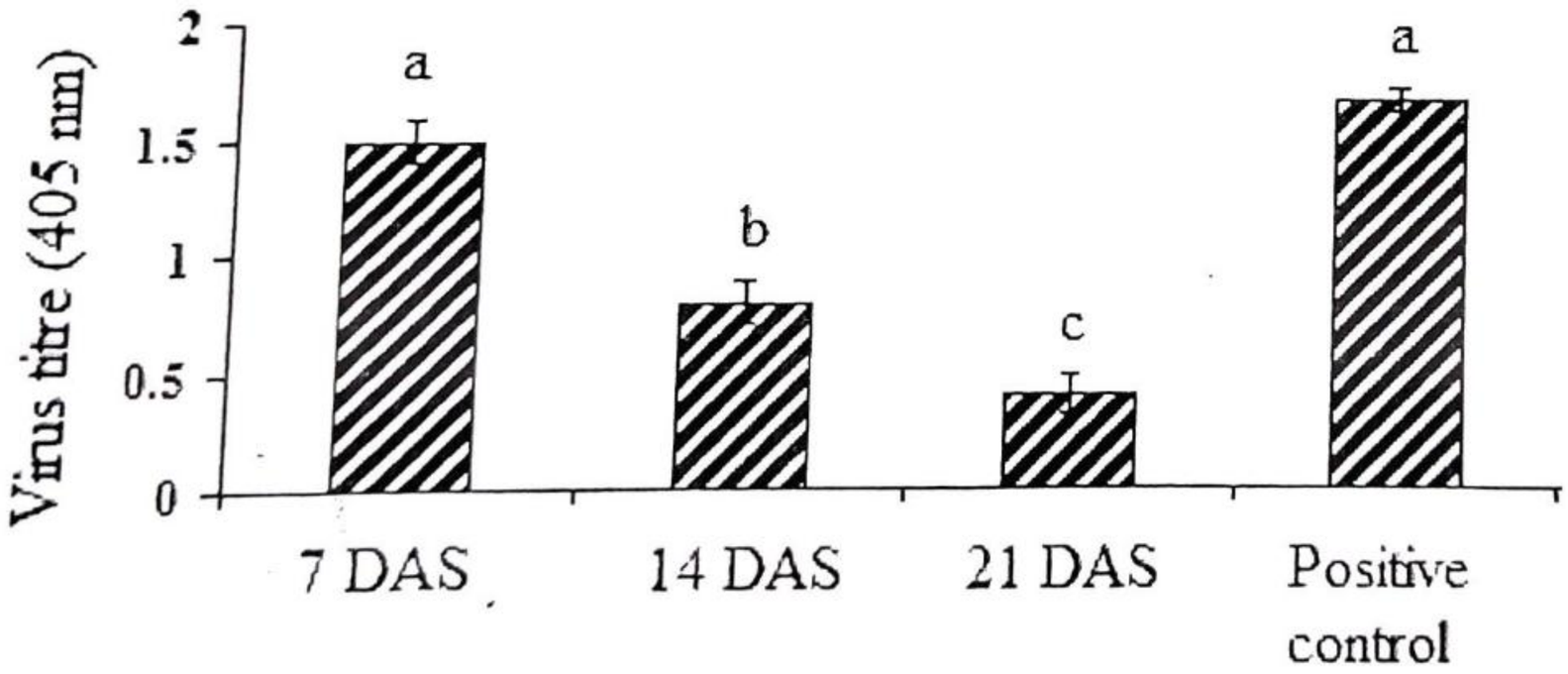

Fig. 2: Relative virus concentration in the leaves of okra plants inoculated with *Cucumber mosaic virus* at different days after sowing (DAS) during the 2014 cropping season in Minna
Vertical bars represent standard deviation

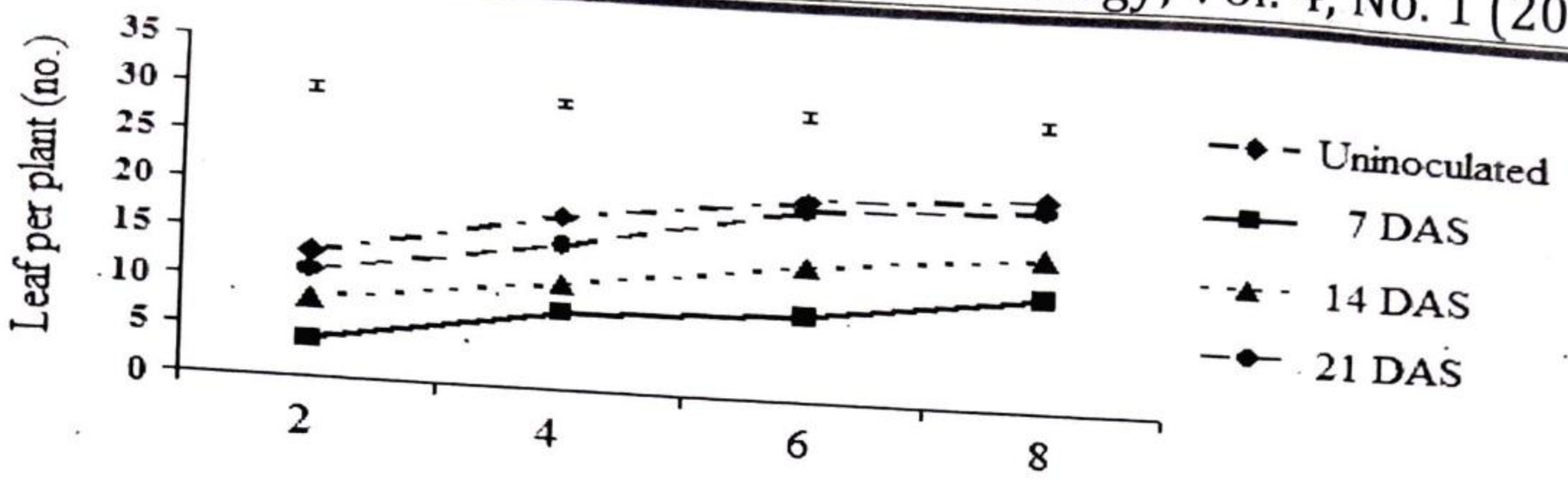

Fig. 3: Number of leaves per plant in okra plants inoculated with *Cucumber mosaic virus* at different days after sowing (DAS) and uninoculated plants during the 2014 cropping season in Minna
Vertical bars represent LSD values

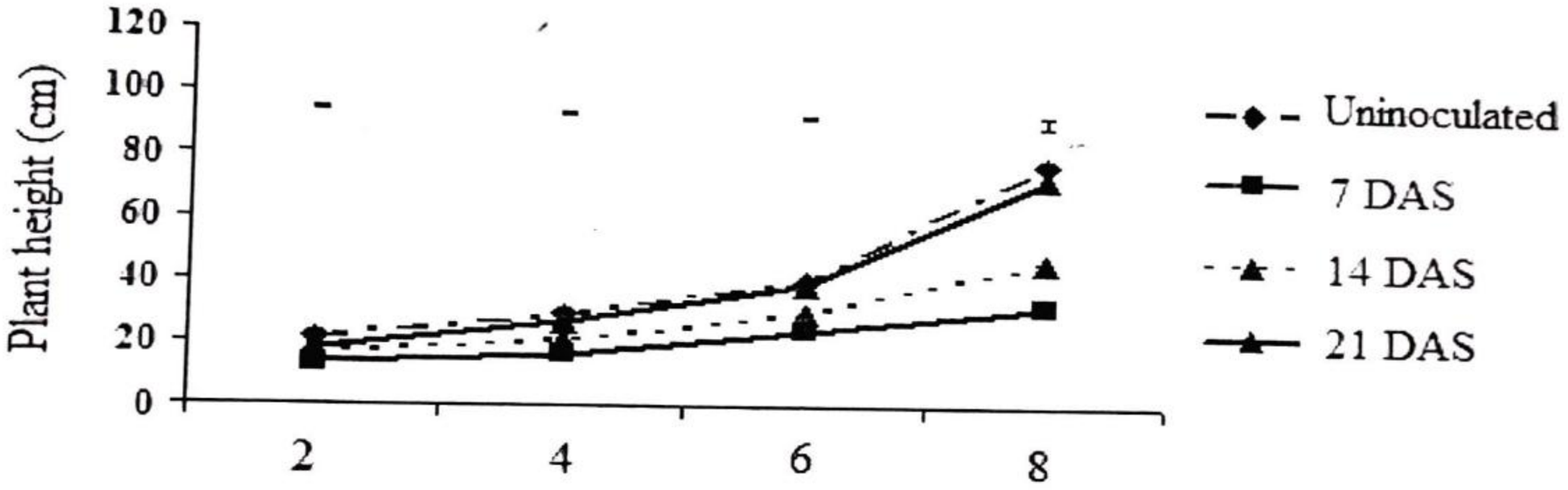

Fig. 4: Plant height from okra plants inoculated with *Cucumber mosaic virus* at different days after sowing (DAS) and uninoculated plants during the 2014 cropping season in Minna
Vertical bars represent LSD values

Table 1: Yield parameters from okra plants inoculated with *Cucumber mosaic virus* at different days after sowing (DAS) and uninoculated plants during the 2014 cropping season in Minna

Treatment	Fruit per plant (no.)	Fruit diameter (cm)	Fruit length (cm)	Fruit weight per plant (g)
Uninoculated	21[a]	2.0[a]	10.0[a]	58.5[a]
7 DAS	8[c]	0.4[c]	5.5[c]	23.5[c]
14 DAS	15[b]	1.0[b]	8.5[b]	36.5[b]
21 DAS	21[a]	2.0[a]	10.0[a]	57.5[a]
±SEM	0.31	0.03	0.17	0.49

Means with dissimilar letter within the column differ significantly at p=0.05 according to Least Significant Difference (LSD)

DISCUSSION

The observation that all the inoculated plants were infected revealed that the okra cultivar "Ikeregi" usedwas susceptible to CMV infection. With respect to the differences in symptoms expression relative to time of infection, the observations reported in this study were in agreement with those documented by Aliyu *et al.* (2010)when cowpea seedlings were infected with a virus inducing yellow mosaic disease of *Commelina benghalensis* L. The highest disease severity observed in the plants inoculated at 7 DAS might be due to low level of resistance to infection at that growth stage. Conversely, the plants inoculated at 21 DAS probably inhibited virus replication and movement. Studies have shown that although virus particles tend to multiply in infected plant cells and consequently cause some physiological changes (Afreen *et al.*, 2011), these become difficult when plants are infected at the later growth stage owing to lignifications of their tissues and organs.

In the plants inoculated at 7 DAS, disease severity increased throughout the period of experiment probably because resistance mechanisms were not activated upon virus entry. Disease severity declined with time in the plants infected at 14 DAS and 21 DAS, indicating that time of infection could influence disease progression and vulnerability of okra to CMV. Lazarowitz (2002) elucidated that although an invading virus may be able to replicate within a single cell, intercellular movement is mediated by the movement proteins (MPs). The MPs accomplish this by modifying pre-existing pathways in the plant for macro-molecular movement in order to facilitate cell-to-cell movement of the viral material. The trend of virus concentration indicated that there was a positive correlation between visual symptom scoring and serological test. This corroborated the results published by Salaudeen *et al.* (2011) when maize plants were infected with *Maize streak virus* (MSV).

The observed reductions in leaf number were an evidence of the relative ability of CMV to cause disease in susceptible plants. Also, the reductions observed in number of leaves per plant supported the findings of El-Dougdoug *et al.* (2007). The devastating effect of CMV on the height of plants infected at 7 DAS agreed with the findings of Aliyu *et al.* (2010). Similarly, Balogun (2008)reported significant growth reductions in tomato seedlings infected with *Potato virus X* and *Tomato mosaic virus* at the early growth stage.Height impairment might be consequences of infection on root development which culminated in reduced moisture and nutrients absorption required for photosynthesis. Plant viruses including CMV have been reported to inhibit the growth and development by altering plants' physiology in vulnerable cultivars(Pallas and Garcia, 2011).

The adverse effects of CMV on the yield parameters of plants infected at 7 and 14 DAS were similar to the observations-reported by Sevik (2011) when pepper (*Capsicumannuum*) plants were infected with *Pepper mild mottle virus*. The general poor performance of the plants infected with CMV at 7 DAS was probably due to accumulated effects of infection on growth parameters, particularly leaf number and plant height. The growth and yield attributes of the plants infected at 21 DAS were not significantly ($p>0.05$) different from uninoculated plants, indicating that physiological processes were mildly affected in the former.

CONCLUSION

The results of this study revealed that growth and yield of okra could be drastically impaired when plants areinfected with CMV at the early growth stage. Therefore, okra farmers should implement adequate measures so as to reduce the effect of viruses infecting okra at early stages of growth. However, integrated pest management strategy such as cultivation of tolerant varieties and use of close spacing could be incorporated as control strategies.

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