TOLERANCE LEVELS OF COWPEA (*Vigna unguiculata* L. Walp) CULTIVARS TO CUCUMBER MOSAIC DISEASE IN MINNA, NORTHERN NIGERIA

Abdulrahman*, A., Salaudeen, M. T. and Gana, A. S.

Department of Crop Production, Federal University of Technology, Minna, Niger State, Nigeria

*Corresponding author: rahmankadir218@gmail.com

ABSTRACT

Cowpea (*Vigna unguiculata*) is a major legume food source for millions of people and feed for livestock in sub-Saharan Africa, Nigeria inclusive. It is also incorporated into traditional cropping system owing to its ability to fix atmospheric nitrogen for soil fertility improvement. Despite its numerous uses, cowpea productivity is threatened by *Cucumber mosaic virus* (CMV) disease. Therefore, eight cowpea cultivars (IT07K-243-1-2, IT07K-298-15, IT07K-303-1, IT08K-126-19, IT08K-150-12, IT08K-180-11, IT08K-193-14 and IT10K-837-1) were tested for CMV disease resistance in Minna, northern Nigeria during the 2016 cropping season. The eight cultivars which constituted the treatments were evaluated as infected and uninfected (control) using randomised complete block design with three replications. Cowpea plants were infected with CMV through mechanical inoculation at 10 days after sowing. All the CMV-infected plants exhibited mosaic and leaf curling symptoms. Disease incidence varied from 81.7 to 96.3 % at 2 weeks after inoculation. The highest number of leaves per plant (112), branches (5) and pod number (2) were observed in the infected plants of IT10K-837-1. On the other hand, the diseased plants of IT07K-243-1-2 produced the highest pod (4.1 g) and seed weight (4.0 g) per plant. Therefore, the IT10K-837-1 could be regarded as the best cultivar for hay and fodder production. Conversely, IT07K-243-1-2 was identified as the promising cultivar for seed production. These two cowpea cultivars could be explored for breeding CVM tolerant cowpea germplasm in order to reduce malnutrition and food insecurity.

Keywords: *Cucumber mosaic virus*, disease incidence and severity, pathogenicity, growth and yield, cowpea cultivars

1 INTRODUCTION

Cowpea (*Vigna unguiculata* L. Walp) is a major leguminous crop grown in Africa (Timko *et al*., 2007). The largest cowpea production is in the moist and dry savannas of sub-Saharan Africa, where it is intensively grown as an intercrop with other cereal crops like millet, sorghum and maize due to the advantages of residual nitrogen, emanating from the decay of roots and root nodules (Ishiyaku *et al.*, 2010). Cowpea has the capability to improve nitrogen content of its immediate environment for growth and development (Ajeigbe *et al.*, 2005). Cowpea seed contains 24 % crude protein, 53 % carbohydrate and 2 % fat (FAO, 2010).

According to IITA (2015), more than 5.4 million tonnes of cowpeas are produced worldwide, with Africa producing about 5.2 million. Investigations have shown that Nigeria is the largest consumer of cowpea worldwide. However, the country produces about 61 % of the cowpea production in Africa. Other prominent cowpea producing countries are Niger, Senegal, Ghana, Mali and Burkina Faso taking the lead (FAO, 2010). Cowpea is infected by about 20 viruses worldwide and nine of them have been reported in Africa (Arogundade *et al.*, 2010). Estimated yield losses as a result of virus infections on the crop vary between 10 and 100 % (Damiri *et al*., 2013). Different viruses are experimentally infectious on cowpea and are termed potential natural threats to cowpea production in the tropics (Alegbejo, 2015). *Cucumber mosaic virus* (CMV) is one of the major viruses infecting legumes (Mih *et al.*, 1991). Studies have shown that CMV disease causes substantial yield losses in several annual crops in Argentina, France, US and some African countries (Nault *et al.*, 2006). Both the quality and quantity of infected plants are seriously impaired. However, the level of CMV pathogenicity is partly influenced by plant's genetic background.

Cucumber mosaic virus is a member of the genus *Cucumovirus* in the family *Bromoviride*. The symptoms of CMV disease vary from slight to severe mosaic, mottling, distortion of leaves and stunting. The virus has a wide host range and attacks a greater variety of vegetables, ornamentals, weeds and other plants including cowpea (Crescenzy, 2003). Knowledge of the incidence and severity of CMV infection would be valuable for genetic improvement of cowpea germplasm. Such information could be utilized in breeding cowpea cultivars with appreciable level of resistance to the virus. Cultivation of CMV- resistant/tolerant varieties would in turn enhance food security. Therefore, this study was conducted to determine the severity of CMV on selected cowpea cultivars in Minna, northern Nigeria.

2 MATERIALS AND METHODS

2.1 Description of the Study Location

The experiment was conducted at the Teaching and Research Farm of the Department of Crop Production, Federal University of Technology, Minna (9^o 51′N, 6^o 44′E and 212 m above sea level), Nigeria during the 2016 cropping season. Minna is located in the Southern Guinea Savanna agro-ecological zone of Nigeria with a mean annual rainfall of 1200 mm (Adeboye *et al*., 2011). The rainfall normally begins in April and ends in the first week of October. The temperature ranges between 35 and 37.5 ^oC, with relative humidity between 40 and 80 %. The soils of Minna originated from basement complex rocks and are generally classified as Alfisols (Adeboye *et al*., 2011).

2.2 Source of Cowpea Seeds

Eight cowpea cultivars (IT07K-243-1-2, IT07K-298-15, IT07K-303-1, IT08K-126-19, IT08K-150-12, IT08K-180-11, IT08K-193-14 and IT10K-837-1) were collected from the cowpea germplasm, International Institute of Tropical Agriculture (IITA), Ibadan, Oyo State, for the study. The evaluated cultivars were selected from the cowpea germplasm commonly grown in the Guinea Savanna of Nigeria.

2.3 Treatment and Experimental Design

The eight cowpea cultivars (treatments) were arranged in a randomised complete block design with three replications. Each cultivar was evaluated in two ridges. The gross plot for each independent trial was 140.25 m^2 and the net plot measurement was 101.25 m^2.

2.4 Source of Virus Inoculum, Multiplication and Inoculation

The CMV isolate used was obtained from the Department of Crop Production, Federal University of Technology, Minna. Virus extract was extracted for inoculation by grinding (1 g/ mL) in extraction buffer containing 0.1M sodium phosphate dibasic, 0.1M potassium phosphate monobasic, 0.01M ethylene diamine tetra acetic acid and 0.001M L-cystine per litre of distilled water, using a pre-cooled sterilized mortar and pestle. Two microlitres of β- mercapto-ethanol were added to the extract just before used.

Cowpea seedlings were infected with CMV inoculum at 10 days after sowing by rubbing the virus extract on the upper surface of the leaves dusted with carborundum powder (600-mesh). The inoculated plants were rinsed with sterile distilled water so as to prevent leaf shading by the powder. Symptomatic leaves were collected from the infected plants at 3 weeks after inoculation (WAI) for inoculation during the main experiment. Infected leaves were preserved at room temperature, in airtight vial bottles on silica gels covered with a thin layer of non-absorbent cotton wool.

2.5 Crop Establishment and Management

The study site was manually cleared of the previous plant remains and ridged in the second week of August, 2016. Cowpea seeds were sown on 19th August, 2016 after the land preparation. Three cowpea seeds were sown per stand after dressing with Apron-plus (metalaxyl+carboxin+furathiocarb) at the rate 3 g per 10 kg. Sowing was done at inter- and intrar- row spacing of 0.75 × 0.30 m, respectively. Seedlings were thinned to one per stand at 8 days after sowing. The CMV infected cowpea leaves previously preserved on silica gels were used for inoculation. Virus extract preparation, time of inoculation and inoculation procedure were as in the preceding sub-section. Weeds were manually controlled through hand weeding at 4 and 6 weeks after sowing (WAS). Insect pests were controlled by spraying D-D force (Cypermethrin plus Dimethoate) at the rate of 200 mL/15L of water. Insecticide was sprayed four times at weekly interval, commencing from 3 WAS. Harvesting was done when pods were dry.

2.6 Data Collection and Statistical Analysis

Data were collected on CMV disease incidence, disease severity, plant height, number of leaves per plant, number of branches per plant, number of days to 50 % flowering, number of pods per plant, pod and seed weight per plant. Disease severity was evaluated using a 1 – 5 scoring scale (Arif and Hassan, 2002). On the scale, 1 = no symptoms, 2 = slight mosaic; 3 = moderate mosaic, 4 = severe mosaic, leaf distortion and stunting, 5 = severe mosaic, stunting and plant death.

The data were subjected to analysis of variance (ANOVA) using the general linear model (PROC GLM) procedure of SAS (2008). The means of significant ($p \leq 0.05$) F-test were separated using the Least Significant Difference (LSD) at 5 % probability level.

3 RESULTS AND DISCUSSION

3.1 Disease incidence and severity

Disease symptoms were first sighted at 10 days after inoculation (DAI). These symptoms were mild leaf chlorosis and mosaic. The differences in disease incidence among the cultivars were not significant ($p>0.05$) (Figure 1A) but the highest was observed in IT08K-180-11 (93.3 %). This was followed by IT07K-243-1-2 and IT07K-303-1 which had 85 % disease incidence. Similarly, IT07K-298-15 and IT08K-193-14 exhibited 81.7 % level of infection. In IT08K-126-19 and IT10K-837-1, disease incidence of 80 % was found, whereas IT08K-150-12 exhibited the lowest disease incidence (75 %). At 2 WAI, percentage of infection varied between 81.7 % in IT08K-150-12 and 96.3 % in IT07K-243-1-2. High disease incidence was also observed in IT07K-303-1 (95 %), IT08K-180-11 (95 %), IT08K-126-19 (88.3 %), IT08K-193-14 (88.3 %), IT10K-837-1 (87.5 %) and IT07K-298-15 (85 %).

The differences in disease severity were also not significant ($p>0.05$) among the cowpea cultivars evaluated (Figure 1B). The symptoms observed varied from mild to moderate level of infection. At 2 WAI, the highest level of disease severity (2.5) was observed in IT10K-837-1 (Fig. 1B). This was followed by IT07K-243-1-2, IT07K-298-15, IT07K-303-1, IT08K-126-19 and IT08K-150-12 which elicited disease severity score of 2.3. Next were IT08K-180-11 and IT08K-193-14 which had severity score of 2.0. At 4 WAI, IT10K-837-1 showed the highest disease severity (2.7), followed by IT07K-243-1-2, IT07K-303-1, IT08K-126-19, IT08K-150-12 and IT08K-193-14 which exhibited disease severity of 2.6 each. The cultivars IT07K-298-15 and IT08K-180-11 elicited symptom score of 2.3.

3.2 Effect of C*ucumber mosaic virus* disease on plant height

Generally, CMV disease caused significant ($p<0.05$) reduction in the height of infected plants, whereas the healthy plants showed rapid growth. The infected plants exhibited poor growth and low vigour. and some of them were characterized by small stems and short internodes at 8 WAI (Figure 2). The height of healthy plants varied between 39.0 cm (IT10K-837-1) and 43.1cm (IT08K-150-12) while infected plants had between 28.0 cm (IT10K-837-1) and 33.0 cm (IT08K-150-12). Height reduction was highest in IT07K-243-1-2 (30.1 %) and the lowest was found in IT08K-126-19 (22.5 %) and IT08K-180-11 (22.5 %).

3.3 Effect of *Cucumber mosaic virus* infection on number of leaves per plant

The number of leaves varied significantly ($p<0.05$) between healthy and infected plants of all the cultivars (Figure 3A). Number of leaves in healthy plants ranged between 145 (IT08K-180-11) and 151 (IT10K-837-1) while in the infected plants it varied between 99 (IT07K-298-15) and 112 (IT10K-837-1). At 8 WAI, reduction in number of leaves per plant was most pronounced in IT07K-298-15 (32.1 %), followed by the cultivar IT07K-243-1-2 which exhibited 31.2 % leaf reduction. In the remaining cultivars reduction in number of leaves ranged from 23.7 to 27.3 % per plant

3.4 Effect of *Cucumber mosaic virus* disease on number of branches per plant

As in other growth parameters, the number of branches per plant differed significantly ($p<0.05$) between healthy and infected plants except in IT07K-243-1-2, IT07K-303-1 and IT08K-150-12 (Figure 3B). The number of branches per plant in healthy plants varied between 6 (IT07K-243-1-2, IT07K-303-1, IT08K-150-12 and IT10K-837-1) and 7 (IT07K-298-15, IT08K-180-11 and IT08K-193-14) while that of infected plants ranged from 4 (IT08K-126-19 and IT10K-837-1) to 5 (IT07K-243-1-2, IT07K-298-15, IT07K-303-1, IT08K-150-12, IT08K-180-11 and IT08K-193-14). Reduction in number of branches per plant was most conspicuous in IT10-837-1 (33.4 %). The cowpea cultivars IT07K-243-1-2, IT07K-303-1 and IT08K-150-12 had 16.7 %) reduction in number of branches per plant. On the other hand, IT08K-298-15, IT08K-180-11 and IT08K-193-14 exhibited 28.6 % reduction in number of branches per plant.

3.5 Effect of *Cucumber mosaic virus* infection on number of days to 50 % flowering

Number of days to 50 % flowering was significantly ($p<0.05$) earlier in the healthy plants compared to those infected with CMV in all the cultivars (Figure 4A). Days to 50 % flowering varied between 46 (IT08K-193-14) and 56 (IT07K-837-1) in the healthy plants, whereas the infected plants had a range of 52 (IT10K-837-1) to 57 (IT08K-193-14) days. Number of days to 50 % flowering was most prolonged in IT08K-193-14 (11 days), whereas IT08K-180-11 and IT10K-837-1 in which 50 % flowering was delayed for 4 days were the least affected.

3.6 Effect of *Cucumber mosaic virus* disease on number of pods per plant

Cucumber mosaic virus disease reduced number of pods per plant significantly ($p<0.05$) (Figure 4B). The healthy plants produced between 5 (IT07K-298-15 and IT07K-303-1) and 11 (IT08K-180-11 and IT08K-193-14) pods per plant while the infected plants had between 1 (IT07K-243-1-2, IT07K-303-1, IT08K-126-19, IT08K-150-12, IT08K-180-11 and IT08K-193-14) and 2 (IT07K-298-15 and IT10K-837-1) pods per plant. IT07K-298-15 and IT10K-837-1 had 60 and 72.6 % reduction in number of pods per plant, respectively. Conversely, pod reduction varied from 80 to 90.6 % among the remaining cultivars.

3.7 Effect of *Cucumber mosaic virus* infection on pod weight per plant

There was a significant ($p<0.05$) difference in pod weight between healthy and CMV-infected plants of all the cultivars. The pods of some of the severely infected cowpea plants were markedly small and deformed contrary to the big and normal shaped pods of healthy plants. The data on pod weight per plant are presented in Figure 5A. The healthy plants produced pod weight which varied from 6.7 (IT70K-303-1) to 23.0 g (IT08K-180-11) while the infected plants had between 2.5 (IT08K-126-19) and 4.1 g (IT07K-243-1-2) per plant. Pod weight reduction was highest in IT08K-180-11 (84.7 %) and the lowest was found in IT07K-298-15 (53 %).

3.8 Effect of *Cucumber mosaic virus* disease on seed weight per plant

All the cultivars exhibited significant ($p<0.05$) difference in seed weight between healthy and infected plants. The virus reduced seed weight at varying levels among the cultivars (Figure 5B). Many of the infected plants produced small seeds while the healthy plants produced large seeds. The seed weight of healthy plants ranged from 3.8 (IT07K-303-1) to 12.0 g (IT08K-193-14) while the seed weight of infected plants varied between 2.1 (IT08K-126-19) and 4.0 g (IT07K-243-1-2). Reduction in seed weight was highest in IT08K-180-11 (78.2 %) while IT07K-243-1-2 had the lowest (23 %).

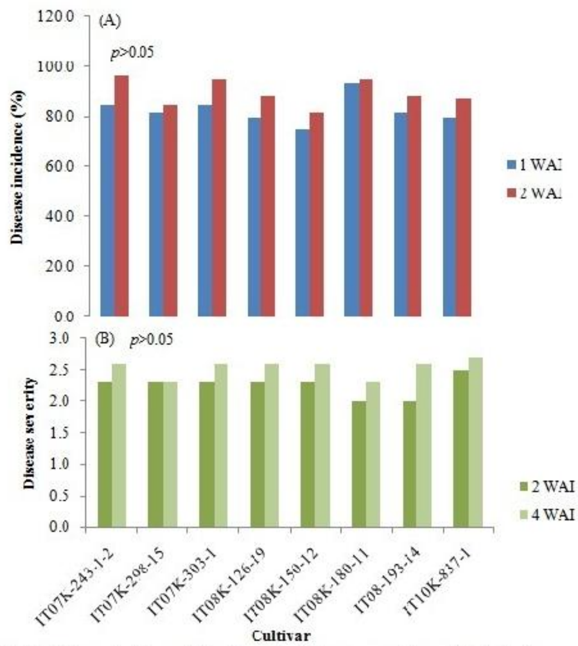

Figure 1: Disease incidence (A) and severity (B) in cowpea cultivars infected with *Cucumber mosaic virus* disease

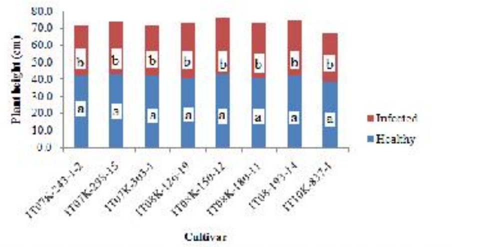

Figure 2 : Plant height from healthy and *Cucumber mosaic virus* infected cowpea cultivars
Bars with dissimilar letter differ significantly at $p=0.05$ according to the Least Significant Difference (LSD)

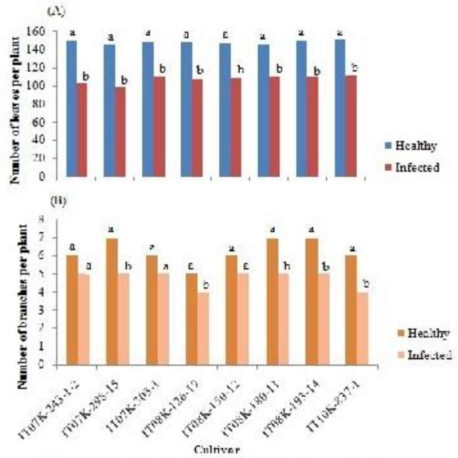

Figure 3 : Number of leaves (A) and branches (B) per plant in healthy and *Cucumber mosaic virus* infected cowpea cultivars
Bars with dissimilar letter differ significantly at $p=0.05$ according to the Least Significant Difference (LSD)

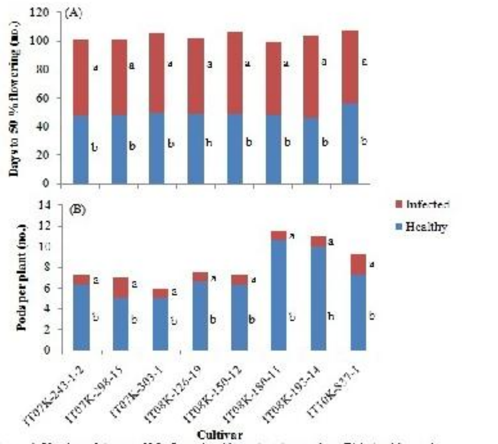

Figure 4: Number of days to 50 % flowering (A) and pods per plant (B) in healthy and *Cucumber mosaic virus* infected cowpea cultivars
Bars with dissimilar letter differ significantly at $p=0.05$ according to the Least Significant Difference (LSD)

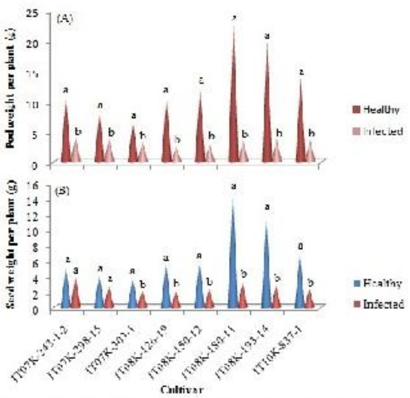

Figure 5 : Pod weight (A) and seed weight (B) per plant in healthy and *Cucumber mosaic virus* infected cowpea cultivars
Cones with dissimilar letter differ significantly at $p=0.05$ according to the Least Significant Difference (LSD)

4 DISCUSSION

Cucumber mosaic virus disease causes significant yield losses in cowpea and several crops of economic importance. The symptoms observed on the infected plants are in agreement with those reported by Arogundade *et al*. (2010). The mosaic symptoms observed on the infected plants revealed that none of the cowpea cultivars was completely resistant to the virus. This is in consonance with the findings of Arogundade *et al*. (2010). Disease severity was variable among the cowpea cultivars owing to the differences in their genetic background. The significantly higher morphological and yield performance of the healthy than infected plants are in agreement with that reported by Balogun and Fawehinmi (2008) when some eggplants were challenged with CMV.

Similarly, the higher number of leaves in the healthy than infected plants is in tandem with the findings of Pazarlar *et al*. (2013). The variability in reductions of the growth and yield parameters is an indication of the differences in cultivars' genetic architecture. This corroborates the findings of Paga'n *et al* (2008), who recorded substantial negative consequences in the CMV infected *Arabidopsis thaliana*. The deleterious effect of CMV disease could impact negatively on cowpea productivity and food security. Seed weight is an important character in cowpea breeding and selection normally favours varieties with appreciable yield (Aliyu and Makinde, 2016). None of the cowpea cultivars exhibited consistent performance for the morphological and yield attributes probably due to different gene actions required for each plant trait. Studies have shown that quantitative traits are controlled by two or more genes which may operate synergistically or antagonistically (Malmberg *et al*., 2005). In spite of this, the cowpea cultivar IT10K-837-1 could be regarded as the best cultivar for hay and fodder production. Conversely, IT07K-243-1-2 was identified as the most promising cultivar for seed production.

5 CONCLUSION AND RECOMMENDATION

This study has established the virulence and pathogenicity of CMV on the evaluated cowpea germplasm. Moreover, the results obtained revealed that the best cowpea cultivars for fodder (IT10K-837-1) and seed (IT07K-243-1-2) production were the most tolerant to CMV infection. Cultivation of these varieties is recommended in the CMV endemic areas in order to reduce malnutrition and food insecurity.

REFERENCES

Adeboye, M. K. A., Bala, A., Osunde, A. O., Uzoma, A. O., Odofin, A. J., & Lawal, B. A. (2011). Assessment of soil quality using soil organic carbon and total nitrogen and microbial properties in tropical agroecosystem. *Agricultural Science,* 2, 34-40.

Ajeigbe, H. A., Singh, B. B. & Oseni, T. O. (2005). Cowpea-cereal intercrop productivity in the Sudan savanna zone of Nigeria as affected by planting pattern, crop variety and pest management. *African Crop Science Journal*, 13(4), 269 – 279.

Alegbejo, M. D (2015). Virus and virus-like diseases of crops in Nigeria. Zaria, Nigeria. Ahmadu Bello University Press. 273pp

Aliyu, O. M. & Makinde, B. O. (2016). Phenotypic analysis of seed yield and yield components in cowpea (Vigna unguiculata L., Walp). *Plant Breeding and Biotechnology*, 4(2), 252 -256.

Arif, M. & Hassan, S. (2002). Evaluation of resistance in soybean germplasm to *Soybean mosaic potyvirus* under field conditions. *Online Journal of Biological Science*, 2, 601-604

Arogundade, O., Balogun, O. S., Shokalu, O & Aliyu, T. H (2010). Influence of *Cowpea mottle virus* and *Cucumber mosaic virus* on the growth and yield of six lines of soybean (*Glycine max* L.Merrril). *Journal Agricultural Science*, 2, 72–78.

Balogun, O. S. and Fawehinmi, O. A. (2008). Influence of seedling age at infection and watering frequency on growth and yield responses of eggplant to *Cucumber mosaic virus*. *African Journal of General Agriculture*. 4, 195–201.

Crescenzy, M. J. (2003). Rearrangements in the 5' nontranslated region and phylogenetic analyses of *Cucumber mosaic virus* RNA 3 indicate radial evolution of three subgroups. *Journal of Virology*, 73, 6752-6758.

Damiri, B. V., Al-Shahwan, I. M., Al-Saleh, M. A., Abdalla, O. A & Amer, M. A. (2013). Identification and characterization of *Cowpea aphid-borne mosaic virus* isolates in Saudi Arabia *Journal of Plant Pathology* 95, (1), 79-85

FAO (Food and Agriculture Organization) (2010). Faostat. Available at http:// Fao.org/site/339/ default. Asp.

IITA (International Institute of Tropical Agriculture) (2015). Cowpea production in Nigeria Available at https://agronigeria.ng>Research.

Ishiyaku, M. F., Higgins, T. J., Umar, M. L., Misari, S. M., Mignouna, H. J., Nang'Ayo, F., Stein, J., Murdock, L. M., Obokoh, M. & Huesing, J. E. (2010). Field evaluation of some transgenic *Maruca* resistant Bt cowpea for agronomic traits under confinement in Zaria, Nigeria. Book of Abstracts of 5th World Cowpea Conference, Dakar, Senegal, pp. 36-37

Malmberg, R. L., Held, S., Waits, A. & Mauricio, R (2005). Epistasis for fitness-related quantitative traits in Arabidopsis thaliana grown in the field and in the greenhouse. *Genetics*, 171, 2015-2027.

Mih, A. M., Atiri, G. I. & Thottappilly, G. (1991). Relationships between co-infection between *Cowpea aphid-borne* and *Cucumber mosaic viruses* and yield of cowpea lines with varying resistance to these viruses. *Phytoparasitica*, 19, 65-72.

Nault, B. A., Shah D. A. & Taylor A. G. (2006). Viruses and aphids everywhere in New York snap bean fields in 2005, 74-76. In: proceedings of the 2006 Empire State Fruit and vegetable EXPO. February 13-16, 2006. Cornell Cooperation Extension, Syracuse, NY. Nigeria IITA pp 143-146

Paga'n, I., Alonson-Blanco, C. & Garci'a-Arenal, F. (2008). Host responces in life-history traits and tolerance to virus infection in *Arabidopsis thaliana*. *PLoS Pathog*. 4:e1000124.

Pazarlar, S., Gumus, M. & Oztekin, G. B. (2013). The effects of tobacco mosaic virus infection on growth and physiological parameters in some pepper varieties (*Capsicum annuum* L.). *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 41, 427-433.

SAS (Statistical Analysis System). (2008). Statistical Analysis System SAS/STAT User's guide, ver.9.2. SAS Institute Inc., Cary, N.C.

Timko, M. P., Ehlers, J. D. & Roberts, P. A. (2007). Cowpea. In: Pulses, Sugar and Tuber Crops, Genome Mapping and Molecular Breeding in Plants (Kole C, ed.). (3). Berlin, Heidelberg Springer-Verlag. 49-67.