RELATIVE TOLERANCE OF SOME COWPEA (*VIGNA UNGUICULATA* L. WALP.) CULTIVARS TO *BLACKEYE COWPEA MOSAIC* AND *COWPEA MOTTLE VIRUSES*

***Ahmed[1], A.A., M. T. Salaudeen[2], M. G. M. Kolo[2] and H. Ibrahim[2]**

[1]Department of Pest Management Technology, Niger State College of Agriculture, Mokwa
[2]Department of Crop Production, Federal University of Technology Minna, Nigeria
*Corresponding author: ahmadkinah68@gmail.com (07030576771)

SUMMARY

Twenty five cowpea cultivars were evaluated for tolerance to *Blackeye cowpea mosaic virus* (BICMV) and *Cowpea mottle virus* (CPMoV) under field conditions at Mokwa, Southern Guinea agro-ecological zone of Nigeria in the 2017 wet season. The trial was laid out using randomized complete block design with three replications. Each of the twenty five cultivars was inoculated singly with BICMV or CPMoV at ten days after sowing (DAS) while for the mixed virus inoculation: each of the twenty five cultivars was inoculated with BICMV or CPMoV at 17 DAS. Parameters recorded include plant height, number of leaves, pods and seeds per pod. Symptom expression such as bright yellowing, mosaic and mottling started one week after inoculation (WAI) from both treatments. At 2 WAI, 85 - 100 % infection was observed among the viruses and cowpea cultivars. The plants showed typical symptoms of both viruses but at varying levels. The rating done on a scale of 1 - 5 with respect to symptomatology indicated that the viruses had significant ($p < 0.05$) severe effects on the crops. The severity of the viruses on symptom rating indicated that BICMV caused the highest severity effects in Ife Brown, IT99K – 316 – 2 and IT07K – 211 – 1 - 8 which rated 4.3, 4.3, and 4.3 at 5 WAI respectively and CPMoV elicited the highest severity effects in Ife Brown, IT99K – 316 – 2 and IT06K – 137 – 1 with ratings of 4.6, 4.6 and 4.6 at 5 WAI respectively. However, BICMV disease severity was significantly lowest (1.9) in IT06K – 137 – 1 while cultivars IT04K – 321 – 2 and IT07K – 211 -1 – 8 exhibited significantly low of 1.7 each disease on CPMoV infection. Cultivars IT06K – 137 – 1 (BICMV) and IT04K – 321 – 2 and IT07K – 211 -1 – 8 which elicited the lowest severity scores also had significantly ($p < 0.05$) highest number (11) of pods per plant. The present results indicated the potentials of cultivars IT06K – 137 – 1 (BICMV) and IT04K – 321 – 2 and IT07K – 211 -1 – 8 (CPMoV) as sources of resistance gene (s) based on low disease severity for breeding cowpea against BICMV and CPMoV disease to enhance productivity and food security.

Keywords: *Blackeye cowpea mosaic virus*, Cowpea, *Cowpea mottle virus*, disease incidence and severity.

COWPEA [*Vigna unguiculata*, (L.) Walp] is one of the annual leguminous food crops grown in many parts of the tropics. Cowpea has a wide variety of uses. It is a primary source of plant proteins in human and animal feeds. The percentage nutritional value of cowpea indicated its protein content to be 23%, fats 1.3%, fibre 1.8%, carbohydrate, 67% and water 8 – 9% (Shiringani and Shimele, 2011). The crop also serves as a cover crop important for nitrogen fixation (Ayenlere *et al*., 2012). Majority of people in the developing countries including Nigeria are engaged in cowpea production, as it has the potential to produce reasonably well under conditions that may render other crops unproductive. But productivity has been very low, less than 200 kg ha^{-1} (Ayenlere *et al*., 2012). This has been attributed to several biotic and abiotic factors (Alegbejo, 2015). The biotic factors that cause yield reduction include insect pests, parasitic weeds as well as virus, fungi and bacterial diseases (Aliyu *et al*., 2012). The abiotic factors include poor soil fertility, drought, heat, acidity and stress due to intercropping with cereals (Ayenlere *et al*., 2012).

There have been reports of *Cowpea mottle virus* (CPMoV), genus *Carmovirus*, and *Blackeye cowpea mosaic virus* (BICMV), genus *Potyvirus* (Aliyu *et al*., 2012) existing on infecting cowpea in Nigeria. Symptoms due to infection by BICMV depends on host cultivar and virus strain (Salaudeen *et al*., 2016). Diseased cowpea plants show variable amount of dark green vein-banding or inter-veinal chlorosis, leaf distortion, blistering and stunting (Arogundade *et al*., 2010). *Cowpea mottle virus* infection is often manifested as bright yellowing (Alegbejo, 2015). In tolerant varieties symptoms usually consist of mottling only. The virus also induces distortion and reduction in leaf size and witches broom syndrome in cowpea.

Virus diseases of cowpea impact more deleterious effects on cowpea production compared to diseases due to fungi, bacteria and nematodes (Agrios, 2005). This study was carried out to investigate the severity of two unrelated viruses on symptoms induced in cowpea and to assess their effects on growth and yield attributes on their hosts.

Materials and Methods

Description of the study location

The experiment was conducted at theTeaching and Research farm of the Faculty of Agriculture, Ahmadu Bello University (ABU) Mokwa Station (09^{O} 18'N and 05^{O} 50'E) situated in the Southern Guinea Savannah agro - ecological zone of Nigeria during the 2016 cropping season. Mokwa has a mean annual rainfall of 1200 mm, which normally begins in April and ends in the first week of October. The temperature ranges between 35 and 37.5 ^{o}C, with relative humidity between 40 and 80 % (Anon, 2016).

Source of cowpea seeds

Twenty five cowpea cultivars (Ife Brown, IT90K – 277 – 2, IT96D – 610, IT97K – 499 – 35, IT97K – 568 – 18,IT97K – 573 – 2 –1, IT98K – 205 –M8, IT98KD – 288, IT99K – 316 – 2, IT99K – 377 – 1, IT00K – 901 – 5, IT03K – 337 – 6, IT04K – 267 – 8, IT04K – 291 – 2, IT04K – 321 – 2, IT04K – 332 – 1, IT06K – 124, IT06K – 137 – 1, IT07K – 211 – 1 – 8, IT07K – 222 – 2, IT07K – 243 – 1 – 10, IT07K – 251 – 3 – 3, IT07K – 292 – 1 – 10, IT07K – 299 – 6, IT07K – 318 – 33) were sourced 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.

Treatments and experimental design

The twenty five cowpea cultivars (treatments) were arranged in a randomized complete block design with three replications. The gross plot for each treatment was 12.5 m × 17.25 m (216 m^2) and the net plot measurement was 3 m × 15.75 m (48 m^2).

Source of virus inoculum, multiplication and Inoculation

The BICMV and CPMoV isolates used were obtained from the Department of Crop Production, Federal University of Technology, Minna. Virus extract was extracted for inoculation by grinding

(1 g/1 mL) the source 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 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 BICMV and CPMoV 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 and monitored for symptom expression. Symptomatic leaves were collected from the infected plant 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.

Crop Establishment and Management

The study site was manually cleared of the previous plant remains and ridged in the second week of August, 2017. Cowpea seeds were sown one week after the land preparation. Three cowpea seeds were sown after dressing with Apron – star (methylthiuram + metalaxyl + carboxin) at the rate 3g per 10kg seeds, in inter- and intra- row spacing of 0.75 and 0.30 m, respectively. Seedlings were thinned to one per stand at 8 days after sowing. The BICMV and CPMoV 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. For the single virus inoculation, each of the twenty five cultivars was inoculated singly with BICMV or CPMoV at ten days after sowing (DAS) while for the mixed virus inoculation: each of the twenty five cultivars was inoculated with double virus mixtures i.e BICMV at 10 days after sowing (DAS) and CPMoV at 17 DAS.

Weeds were manually controlled through hand weeding at 4 and 6 weeks after sowing. Insect pests were controlled by spraying D-D force (Cypermethrin plus Dimethoate) and pods were harvested at physiological maturity.

Data collection and statistical analysis

Disease incidence was recorded at 2 weeks after inoculation (WAI), as percentage of total plants exhibiting symptoms of BICMV, CPMoV and BICMV+ CPMoV infection. Disease severity was taken at 2 WAI. Disease severity assessment was based on percentage of the topmost leaf surface covered with symptom and general appearance of the plants. A visual 1 to 5 scoring scale was used (Arif and Hassan, 2002). On the scale, 1 = leaves without visible symptom; 2 = leaves exhibiting slight mosaic; 3 = distorted leaves with mosaic; 4 = stunted plants with distorted leaves and severe mosaic; and 5 = dead or stunted plants with severe mosaic. Data were also collected on plant growth and yield parameters.

Data were subjected to analysis of variance (ANOVA) using Statistical Analysis System (SAS, 2008) to verify if there were significant differences among the cultivars. Significance was determined at 5 % level of probability. Where the *F*-test ratio was significant, means were separated using Student-Newman-Keuls (SNK) test.

RESULTS

Disease incidence and severity

Disease symptoms were first observed at 10 days after inoculation (DAI). Typical mosaic symptoms of BICMV infection began with intermingled patches of normal and light green colour on the leaves, these symptoms were observed on the third and fourth leaves while the leaves below were symptomless. On the other hand, those inoculated with CPMoV showed formation of irregular pattern of indistinct light and dark spots on leaves which were more pronounced on younger leaves. However, symptoms observed on mixed infection of BICMV + CPMoV did not differ from those of the BICMV and CPMoV infected crops. At 2 WAI, disease incidence was

significantly (p<0.05) highest in BICMV infected Ife Brown cultivar with 82.3 %. This was followed by IT06K-137-1, IT07K-243-1-10 and IT07K-299-6 which had 63.3 % disease incidence. Cultivars IT96D-610, IT97K-499-35, IT99K-316-2, IT99-377-1, IT00K-901-5, IT04-291-2, IT04K-332-1, IT07K-222-2 and IT07K-251-3-3 elicited disease incidence of 56.6 % and IT07K-292-1-10 had 53.3 % incidence whereas IT97K-568-18 and IT06K-124 exhibited the lowest disease incidence of 50 % (Table 1). Percentage of infection also varied between 84.3 % in Ife Brown and 70.0 % in IT07K-318-33 in CPMoV infected cowpea cultivars. Next in high disease incidence were IT07K-299-6 with 63.4 %, IT04K-321-2 with 63.2 %and IT98KD-288 with 61.6 % which were not significantly (p 0.05) different. Cultivars IT97K-499-35, IT99-377-1, IT00K-901-5 and IT06K-124 exhibited 57% level of infection and IT07K-292-1-10 had the lowest incidence of 50 %. Similarly, percentage incidence on BICMV + CPMoV cowpea cultivars did not vary significantly from their counterparts infected with BICMV alone (Table 1).

Disease severity differed significantly (p<0.05) amongst the 25 cowpea cultivars investigated irrespective of the four virus treatments. Disease severity increased progressively after inoculation, at 2 WAI, the symptoms observed on plants inoculated with BICMV + CPMoV were not much different from those of BICMV alone, and the symptoms observed on CPMoV + BICMV were also like those of CPMoV alone (Table 1). Disease severity at 2 WAI was significantly (p<0.05) higher in Ife Brown with 3.6 score, IT97K-568-18, IT06K-124 and IT07K-292-1-10 had a symptom score of 2 in BICMV infected cowpea plants, IT03K-337-6 exhibited a mean severity score of 1.0 and moderate level of severity symptom score of 3.0 was observed in the other cultivars. In CPMoV infected cowpea plants, disease severity ranged between 1.0 and 3.6. The lowest symptom score of 1.0 was observed in IT90K-277-2, IT96D-610, IT04K-332-1 and IT07K-243-1-10, disease severity was mild in IT07K-222-2 and IT07K-292-1-10 with 2.0, while IT03K-337-6, IT04K-291-2, IT04K-321-2, IT06K-124, IT06K-137-1, IT07K-211-1-8, IT07K-251-3-3 and IT07K-299-6 exhibited a mean severity score of 3.0.

Table 1: Incidence and severity of single and mixed infections of *Blackeye cowpea mosaic* virus (BICMV) and *Cowpea mottle virus* (CPMoV) on cowpea plants at Mokwa in 2017

	Disease incidence at 2 WAI (%)				Disease severity at 2 WAI			
Cultivar	BICMV	CPMoV	BI + CP	CP + BI	BICMV	CPMoV	BI + CP	CP + BI
Ife Brown	82.3^{a}	84.3^{a}	78.3^{a}	80.2^{a}	3.6^{a}	3.6^{a}	3.6^{a}	3.6^{a}
IT90K-277-2	61.6^{b}	53.3^{c}	70.0^{abc}	70.0^{abc}	2.6^{abc}	1.0^{d}	3.6^{a}	3.6^{a}
IT96D-610	56.6^{b}	53.3^{c}	53.3^{c}	73.3^{ab}	3.0^{ab}	1.0^{d}	1.0^{b}	3.6^{a}
IT97K-499-35	56.6^{b}	56.6^{c}	53.3^{c}	78.2^{ab}	3.0^{ab}	3.0^{ab}	1.0^{b}	3.0^{ab}
IT97K-568-18	50.0^{b}	50.0^{c}	66.6^{abc}	50.0^{d}	1.6^{cd}	3.0^{ab}	3.0a	2.0^{b}
IT97K-573-2-1	60.0^{b}	60.0^{bc}	53.3^{c}	73.3^{ab}	2.6^{abc}	3.6^{a}	1.0^{b}	3.0^{ab}
IT98K-205-M8	60.0^{b}	60.0^{bc}	73.3^{ab}	73.3^{ab}	3.0^{ab}	2.6^{abc}	3.0^{a}	3.0^{ab}
IT98KD-288	61.6^{b}	61.6^{bc}	56.3bc	73.1^{ab}	3.0^{ab}	3.0^{ab}	3.0^{a}	3.0^{ab}
IT99K-316-2	56.6^{b}	53.3^{c}	70.0^{abc}	70.0^{abc}	2.6^{abc}	1.0^{d}	2.6^{a}	2.6^{ab}
IT99K-377-1	56.6^{b}	56.6^{c}	53.3^{c}	76.6^{ab}	3.0^{ab}	3.0^{ab}	3.0^{a}	3.0^{ab}
IT00K-901-5	56.6^{b}	56.5^{c}	70.1^{abc}	70.0^{abc}	2.6^{abc}	3.0^{ab}	2.6^{a}	2.6^{ab}
IT03K-337-6	60.0^{b}	60.3^{bc}	66.6^{abc}	66.6^{a-d}	1.0^{d}	2.6^{abc}	2.6^{a}	2.6^{ab}
IT04K-267-8	61.6^{b}	53.3^{c}	63.6^{abc}	63.3^{a-d}	3.0^{ab}	1.0^{d}	2.6^{a}	2.6^{ab}
IT04K-291-2	56.6^{b}	56.3^{c}	66.6^{abc}	66.6^{a-d}	3.0^{ab}	3.0^{ab}	3.0^{a}	3.0^{ab}
IT04K-321-2	61.6^{b}	63.2^{bc}	56.6^{bc}	63.3^{a-d}	2.6^{abc}	2.6^{abc}	2.6^{a}	2.6^{ab}
IT04K-332-1	56.6^{b}	53.3^{c}	63.3^{abc}	53.3^{cd}	3.0^{ab}	1.3^{cd}	1.0^{b}	2.0^{b}
IT06K-124	50.0^{b}	56.9^{c}	66.6^{abc}	66.6^{a-d}	2.0^{bcd}	3.3b	3.0^{a}	3.0^{ab}
IT06K-137-1	63.3^{b}	53.3^{bc}	63.3^{abc}	63.3^{a-d}	3.0^{ab}	3.0^{ab}	3.0^{a}	3.0^{ab}
IT07K-211-1-8	60.0^{b}	60.2^{bc}	66.6^{abc}	66.6^{a-d}	2.6^{abc}	3.0^{ab}	2.6^{a}	2.6^{ab}
IT07K-222-2	56.6^{b}	53.3^{c}	60.0^{abc}	60.0^{bcd}	3.0^{ab}	2.0^{bc}	3.0^{a}	2.0^{b}
IT07K-243-1-10	63.3^{b}	53.3^{c}	53.6^{bc}	73.3^{ab}	2.6^{abc}	1.0^{d}	2.6^{a}	2.6^{ab}
IT07K-251-3-3	56.6^{b}	60.4^{bc}	73.3^{ab}	74.0^{ab}	2.6^{abc}	2.6^{abc}	2.6^{a}	2.6^{ab}
IT07K-292-1-10	53.3^{b}	50.0^{e}	56.6^{bc}	63.3^{a-d}	2.3^{bc}	2.0^{bc}	1.0^{b}	1.0c
IT07K-299-6	63.3^{b}	63.4^{bc}	70.0^{abc}	70.0^{abc}	3.0^{ab}	3.0^{ab}	3.0^{a}	3.0^{ab}
IT07K-318-33	60.0^{b}	70.0^{b}	73.5^{ab}	73.3^{ab}	2.6^{abc}	2.6^{abc}	2.6^{a}	2.6^{ab}
± SEM	1.9	0.38	0.21	0.50	1.9	0.2	0.21	0.2

Means with the same letter (s) within the same column are not significantly (p<0.05) different by Student-Newman -Keuls (SNK) test.

The other cultivars Ife brown, IT97K-573-2-1 and IT07K-318-33 had a mean symptom score of 3.6. Generally, disease severity in the mixture of BICMV + CPMoV infected cowpea cultivars did not differ significantly (p>0.05) from those of BICMV infected cowpea cultivars alone (Table 1).

Effect of single and mixed infections with *Blackeye cowpea mosaic* and *Cowpea mottle* on growth parameters

Plant growth parameters were impaired by the viruses and the magnitude of effects varied with cultivars. This study revealed significant (p<0.05) impairments in the plant height of the 25 cultivars of cowpea both in single and mixed infections of the viruses used. The variation in this parameter with respect to BICMV infections at 5 WAI is presented in Table 2. Cowpea plant varied in heights from 20 to 27.1 cm for the BICMV infected plants, 20 to 26.7 cm for CPMoV infected plants, 20 to 27.9 cm for BICMV + CPMoV infected plants. Plant height was highest in BICMV infected IT0K-337-6 cowpea cultivar with 27.1 cm followed by IT07K-292-1-10 with 27 cm, IT97K-568-18 with 26.1 cm and IT06K-124 with 25.6 cm whereas the lowest value was found in IT96D-610 with 20 cm (Table 2). In the plants infected with CPMoV; IT07K-292-1-10 was the only cultivar which produced significantly (p<0.05) tallest plants of 26.7 cm followed by IT04K-332-1 with 26.3 cm and IT07K-222-2 with 26 cm. Cultivars IT99K-316-2, IT07K-243-1-10, IT90K-277-2, IT04K-267-8 and IT06K-124 elicited mean plant heights of 25.7, 25.5, 25.3, 25.2 and 25 cm respectively. Cultivars IT99K-377-1 and IT07K-251-3-3 did not differ (p >0.05) whereas IT07K-318-33 had the lowest mean value of 20 cm (Table 3). In BICMV + CPMoV inoculated cowpea plants, significantly (p<0.05) tallest plants were found in IT98KD-288 with 27.9 cm followed by IT97K-568-18 with 26.3 cm. Cultivars IT06K-124 and IT07K-299-6 had same mean values of 25.6 cm in height. Cultivars IT97k-573-2-1 and IT07K-251-3-3 also exhibited statistically similar mean height of 25.3 and 25 cm respectively whereas the shortest plants were found in IT96D-610 with 20 cm (Table 2).

The twenty five cowpea cultivars exhibited strong variation in crop branch development. Substantial differences were found within the cowpea cultivars with the various virus treatments.

The number of branches of the crops irrespective of all the virus treatments ranged significantly ($p<0.05$) from 0 to 5 (Table 2). Cultivar IT97K-568-18 infected with BICMV had the highest number of branches of 5 per plant followed by IT07K-292-1-10 with 4 branches. Ife Brown, IT90K-277-2, IT96D-610, IT96K-499-35, IT98K-205-M8, IT04K-291-2 and IT07K-318-33 equally produced similar branch numbers of 2 each whereas IT99K-377-1, IT00K-901-5, IT04K-267-8, IT04K-332-1, IT06K-137-1, IT07K-211-1-18 and IT07k-243-1-10 had no branch at all. Similarly, IT90K-277-2, IT99K-316-2 and IT07k-292-1-10 had the highest number of branches of 5 per plant while IT06K-137-1 produced no branch in the CPMoV infected plants (Table 3). Also, cowpea cultivar IT97K-568-18 in the BICMV + CPMoV infection produced significantly (p 0.05) highest number of branches of 4 followed by IT06K-124 and IT07K-292-1-10 with 3 branches which did not differ from one another. The lowest value of 1 branch was found in Ife Brown, IT96D-610, IT97K-573-2-1, IT98K-205-M8, IT98KD-288, IT99K316-2 and IT07K-292-1-10 whereas IT90K-277-2, IT00K-901-5, IT04K-267-8, IT04K-291-2, and IT07K-251-3-3 produced no branch (Table 2).

Table 2: Height and number of branches of cowpea plants infected with single and mixture of *Blackeye cowpea mosaic virus* (BICMV) and *Cowpea mottle virus* (CPMoV) at 6 weeks after inoculation at Mokwa in 2017

	Plant height (cm)				Number of branches			
Cultivar	BICMV	CPMoV	BI + CP	CP + BI	BICMV	CPMoV	BI + CP	CP + BI
Ife Brown	19.7^{d}	22.2^{fgh}	21.0^{fg}	21.3^{fg}	2^{bc}	1^{cde}	1^{c}	2^{a-c}
IT90K – 277 – 2	20.8^{cd}	25.3^{a-d}	21.0^{fg}	23.0^{def}	2^{bc}	4^{a}	0^{c}	2^{a-c}
IT96D – 610	20.0^{d}	23.2^{c-h}	20.0^{g}	24.4^{bcd}	1^{cde}	3^{a-c}	1^{c}	4^{a}
IT97K – 499 – 35	22.4^{bcd}	22.4^{c-h}	22.4^{def}	24.9^{bc}	2^{cd}	1^{cde}	0^{c}	3^{ab}
IT97K – 568 – 18	26.1^{a}	21.2^{h}	26.3^{b}	21.2^{fg}	5^{a}	1^{cde}	4^{b}	0^{c}
IT97K – 573 – 2 – 1	23.3^{b}	23.5^{b-h}	25.3^{b}	27.1^{a}	1^{cde}	1^{cde}	1^{c}	3^{ab}
IT98K – 205 –M8	22.2^{bcd}	22.3^{fgh}	22.3^{def}	22.2^{efg}	2^{cd}	2^{b-e}	1^{c}	1^{a-c}
IT98KD – 288	21.2^{bcd}	21.2^{h}	27.9^{a}	26.1^{ab}	1^{cde}	1^{cde}	6^{a}	3^{ab}
IT99K – 316 – 2	20.8^{cd}	25.7^{ab}c	24.4^{bcd}	21.8^{efg}	1^{cde}	4^{ab}	1^{c}	2^{a-c}
IT99K – 377 – 1	22.2^{bcd}	23.4^{b-h}	22.4^{def}	26.0^{ab}	0^{e}	1^{cde}	1^{c}	3^{ab}
IT00K – 901 – 5	21.2^{bcd}	21.7^{gh}	24.4^{bcd}	21.2^{fg}	0^{e}	0de	0^{c}	0^{c}
IT03K – 337 – 6	27.1^{a}	23.5^{b-h}	23.0^{def}	23.2^{def}	4^{ab}	0de	1^{c}	2 $^{a-c}$
IT04K – 267 – 8	22.2^{bcd}	25.2^{a-e}	23.2^{c-f}	22.2^{efg}	0^{e}	2^{a-d}	0^{c}	0^{c}
IT04K – 291 – 2	21.2^{bcd}	21.2^{h}	23.3^{c-f}	25.7^{ab}	1^{cde}	1^{cde}	0^{c}	3^{a-c}
IT04K – 321 – 2	20.8^{cd}	24.3^{a-f}	24.1 $^{b-e}$	21.8^{efg}	1^{cde}	1^{cde}	1^{c}	1^{bc}
IT04K – 332 – 1	22.6^{b}c	26.3^{ab}	22.8^{def}	25.1^{bc}	0^{e}	3^{abc}	0^{c}	2^{a-c}
IT06K – 124	25.6^{a}	25.0^{a-f}	25.6^{b}	22.2^{efg}	2^{bc}	4^{a}	3^{b}	1^{a-c}
IT06K – 137 – 1	21.2^{bcd}	21.8^{gh}	22.7^{def}	21.3^{fg}	0e	0^{e}	1^{c}	1^{a-c}
IT07K – 211 – 1 – 8	20.8^{cd}	22.7^{ef}	22.3^{def}	21.6^{efg}	0e	0de	1^{c}	1^{bc}
IT07K – 222 – 2	22.2^{bcd}	26.0^{ab}	20.1^{def}	22.7^{def}	1^{cde}	3^{a-d}	0^{c}	0^{c}
IT07K – 243 – 1 – 10	21.2^{bcd}	25.5^{abc}	23.4^{c-f}	25.6^{ab}	0^{e}	2^{a-e}	1^{c}	2^{a-c}
IT07K – 251 – 3 – 3	23.1^{bc}	23.4^{b-h}	25.0^{b}	23.6^{bcd}	1^{cde}	1^{cde}	0^{c}	1^{bc}
IT07K – 292 – 1 – 10	27.0^{a}	26.7^{a}	24.1 $^{b-e}$	25.7^{ab}	4^{ab}	4^{a}	1^{c}	3^{ab}
IT07K – 299 – 6	21.2^{bcd}	21.2^{h}	25.6^{b}	21.5^{efg}	1^{cde}	1^{cde}	3^{b}	1^{bc}
IT07K – 318 – 33	20.8^{cd}	20.0^{h}	21.7^{efg}	20.8^{g}	1^{cde}	1^{cde}	1^{c}	2^{a-c}
± SEM	0.47	0.59	0.6	0.45	0.34	0.38	0.62	0.45

Means with the same letter (s) within the same column are not significantly (p<0.05) different by Student-Newman -Keuls (SNK) test

Table 3: Number of pods per plant and seed numbers per pod in cowpea cultivars infected with single and mixture of *Blackeye cowpea mosaic virus* (BICMV) and *Cowpea mottle virus* (CPMoV) at Mokwa in 2017

	Number of pods/plant				Number of seeds/pod			
Cultivar	BICMV	CPMoV	BI + CP	CP + BI	BICMV	CPMoV	BI + CP	CP + BI
Ife brown	3^{b}	4^{bcd}	1^{c}	2^{c}	2^{c}	1^{b}	1^{b}	2^{fg}
IT90K-277-2	3^{b}	7^{abc}	1^{c}	2c	2^{c}	3^{ab}	2^{b}	2^{fg}
IT96D-610	3^{b}	9^{a}	1^{c}	8^{ab}	1^{c}	4^{a}	1^{b}	5^{b}
IT97K-499-35	1^{b}	2^{d}	1^{c}	7^{ab}	2^{c}	2^{ab}	2^{b}	5^{b}
IT97K-568-18	7^{a}	3^{d}	7^{b}	2^{c}	5^{ab}	2^{ab}	2^{b}	2^{fg}
IT97K-573-2-1	4^{b}	4^{bcd}	3^{c}	9^{ab}	2^{c}	2^{ab}	4^{b}	7^{a}
IT98K-205-M8	2^{b}	2^{d}	1^{c}	2^{c}	2^{c}	2^{ab}	2^{b}	2^{fg}
IT98KD-288	3^{b}	3^{d}	11^{a}	10^{a}	2^{c}	1^{b}	6^{a}	7^{a}
IT99K-316-2	2^{b}	7^{abc}	2^{c}	2^{c}	2^{c}	4^{a}	3^{b}	1^{fg}
IT99K-377-1	2^{b}	2^{d}	1^{c}	2^{c}	1^{c}	2^{ab}	1^{b}	7^{a}
IT00K-901-5	4^{b}	4^{bcd}	1^{c}	4^{c}	2^{c}	2^{ab}	2^{b}	2^{fg}
IT03K-337-6	9^{a}	3^{d}	2^{c}	3^{c}	5^{ab}	2^{ab}	2^{b}	1^{fg}
IT04K-267-8	3^{b}	8^{a}	2c	3^{c}	2^{c}	4^{a}	4^{b}	1^{fg}
IT04K-291-2	3^{b}	3^{d}	2^{c}	10^{a}	1^{c}	3^{ab}	1^{b}	5^{b}
IT04K-321-2	1^{b}	1^{d}	2^{c}	4^{c}	3^{abc}	3^{ab}	3^{b}	2^{fg}
IT04K-332-1	3^{b}	8^{a}	1^{c}	8^{ab}	2^{c}	3^{ab}	2^{b}	5^{b}
IT06K-124	8^{a}	8^{a}	7^{b}	3^{c}	5^{ab}	4^{a}	4^{b}	1^{fg}
IT06K-137-1	2^{b}	2^{d}	2^{c}	1^{c}	2^{c}	3^{ab}	2^{b}	2^{fg}
IT07K-211-1-8	3^{b}	3^{d}	1^{c}	3^{c}	2^{c}	3^{ab}	3^{b}	0^{g}
IT07K-222-2	2^{b}	7^{abc}	2^{c}	3^{c}	1^{c}	4^{a}	1^{b}	2^{fg}
IT07K-243-1-10	2^{b}	7^{abc}	2^{c}	8^{ab}	1^{c}	3^{ab}	1^{b}	5^{b}
IT07K-251-3-3	1^{b}	1^{d}	2^{c}	2^{c}	2^{c}	1^{b}	2^{b}	0^{g}
IT07K-292-1-10	8^{a}	8^{a}	1^{c}	7^{ab}	6^{a}	4^{a}	1^{b}	5^{cde}
IT07K-299-6	4^{b}	4^{bcd}	6^{b}	4^{c}	2^{c}	1^{b}	4^{b}	2^{fg}
IT07K-318-33	2^{b}	2^{d}	2^{c}	2^{c}	1^{c}	2^{ab}	1^{b}	1^{fg}
± SEM	0.75	0.76	1.15	0.69	0.59	0.47	0.6	0.58

Means with the same letter (s) within the same column are not significantly (p<0.05) different by Student-Newman -Keuls (SNK) test.

Effect of single and mixed infections with *Blackeye cowpea mosaic* and *Cowpea mottle virus* on yield and yield components

The difference in number of pods per plant were significantly (p<0.05) different and ranged from 1 to 11 irrespective of all virus treatments among the 25 cultivars. In the BICMV infected plants, IT03k-337-6 produced the highest number of pods per plant of 9 followed by IT06k-124 and IT07k292-1-10 which produced 8 pods per plant whereas the remaining cultivars produced between 1 and 4 pods per plant (Table 5). Cultivar IT96D-610 infected with CPMoV produced the highest number of pods per plant of 9 while cultivars IT04K-267-8, IT04K-332-1, IT06K-124 and IT07K-292-1-10 produced 8 pods per plant. Cultivars IT04K-321-2 and IT07K-251-3-3 gave the least pod per plant of 1. On the hand, BICMV + CPMoV infected IT98KD-288 produced the highest number of pods per plant of 11. Cowpea cultivars IT97K-568-18 and IT06k-124 produced 7 pods per plant and IT07K-299-6 had 6 pods per plant. Ife Brown, IT90K-277-2, IT96D-610, IT97K-499-35, IT98K-205-M8, IT99K-377-1, IT00K-901-5, IT04K-332-1, IT07K-211-1-8 and IT07K-292-1-10 produced an average of 1 pod per plant (Table 5).

Cowpea cultivar IT07K-292-1-10 infected with BICMV recorded the highest number of seeds of 6 per pod followed by IT97K-568-18, IT003K-337-6 and IT06K-124 which produced 5 seeds per pod. The remaining cultivars had number of seeds which did not differ significantly (p>0.05) from one another. Also, CPMoV infection on IT96D-610, IT99K-316-2, IT04K-267-8, IT06k-124, IT07K-222-2 and IT07K-292-1-10 gave the highest number of seeds per pod of 4. In the same virus treatment, cultivar Ife Brown gave the least number of seeds per pod of 1 whereas the remaining cultivars produced number of seeds which did not differ significantly from one another (5). Also, cowpea cultivar IT98KD-288 produced the highest number of seeds per pods (8) in BICMV + CPMoV inoculated plants followed by IT97K-573-2-1, IT04K-267-8, IT06K-124 and IT07K-299-6 that had 4 seeds per pod each which were similar statistically. The remaining cowpea cultivars gave a minimum number of seeds which ranged from 1-3 per pod.

DISCUSSION

Screening of cowpea cultivars for sources of resistant genes has gained importance owing to the severe losses caused by legume viruses (Arogundade *et al*., 2010). The significant differences observed among the tested cultivars in response to BICMV and CPMoV inoculations indicate their genetic diversity. In this study, the result which showed that disease symptoms appeared on the leaves soon after inoculation is consistent with the findings of Aliyu *et al*. (2012) who noted that CPMoV induced mosaic symptom on legume crops in not more than one week when plants were inoculated at the early 10 days post-planting growth stage. Mosaic, mottling, vein banding and chlorosis symptoms were observed first on the topmost leaves because they served as infection points. Cultivars IT97K-568-18, IT04K-321-2, IT07K-292-1-10, IT90K-277-2, IT96D-610, IT99K-316-2 and IT06K-124 among the cowpea cultivars investigated produced moderate symptom of infection at 10 days post-planting suggesting the presence of BlCMV and CPMoV-tolerant genes. However, the fact that complete infection was observed at 2 week after inoculation across the cultivar implies that none of the cowpea cultivars was immune to the two viruses. Immunity is the highest level of resistance and is partly exhibited as absence of visible symptoms in inoculated plants (Salaudeen *et al*., 2016). The differences exhibited with respect to symptom severity could also be attributed to varied inherent genetic constitution of the evaluated cultivars.

Height impairment was high in some cultivars because the virus was more pathogenic on them, unlike their moderately tolerant counterparts. Again, the differences in the magnitude of height impairment or increase among the cultivars investigated could be traced to the differences in their genetic architecture, hyperplasia or hypoplasia. Also, plant height reduction was highest in the BICMV infected Ife Brown and IT07K-318-33 infected with CPMoV probably because of their poor BICMV and CPMoV tolerance constitution. Similarly, due to the genetic differences among the cultivars investigated, the number of branches was highest in the tolerant IT97K-568-18 and IT07K-292-1-10 cultivars. However, the poor genetic make-up of the susceptible cultivars resulted in the production of few or even no branches. Generally, it could be that trait for these

characteristics were inherited along with resistance genes from the breeding process, as evidenced from their heights. There are several reports of genetic linkage where two or more genes are located on the same chromosome, and they tend to move together (Agrios, 2005).

Pod numbers and number of seeds per plant are important attributes for genotype selection in cowpea. Highly susceptible cowpea cultivars in the present study produced few and shriveled pods and seeds this resulted in lower seed yield compared to their tolerant counterparts. The impact of the virus arose from the negative effects associated with infection. Thus in infected plants the proportion of chloroplast may be drastically reduced (Agrios, 2005). Therefore, lower amount of this was translocated to the food reserve in infected plants. Similar to the results of this study, Adamu *et al*. (2015) found lower pod and seed yield in cucumber mosaic virus infected soyabean.

Generally, in this study, the growth and subsequent yield of some cultivars can be likened to both single and double infections suggesting that the response of some of these cultivars to double infections was stronger than the sum of the effects caused by each of the virus in single infection. This concurs with the opinion of Taiwo and Akinjogunla (2006) who reported that double virus infections resulted in greater reduction in the growth and yield of cowpea cultivars. Several studies involving mixed virus infections have demonstrated synergistic interactions (Nsa and Kareem, 2015) in mixed virus infections using growth parameters such as plant height, yield and effect on seeds in cowpea (Mamman *et al.,* 2017).This result support the hypothesis that sequence of infection has marked effect on the virus accumulation dynamics in mixed infection and the eventual synergistic disease development in cowpea pathosystem.

CONCLUSION

The study had demonstrated that variability existed among the twenty-five cowpea cultivars investigated. Complete infection was obtained in the field experiment. Response of these cultivars to single and mixed infections was influenced by their genetic constitution or make-up. Also, the yield and agronomic traits of the cultivars were largely determined by their genetic architecture.

Although there was no immunity to these virus diseases among the populations evaluated, some cowpea cultivars showed appreciable level of tolerance and desirable yield.

The benefit of increased cowpea production include improved nutrition for humans and livestock, improved soil properties and substantial opportunities for greater income. The monitoring and management of these viruses therefore is crucial to sustainable cowpea production most especially in sub-Saharan Africa. Finally, virus-free seed should be produced and supplied to breeders for intensive biotechnological research that will result in the development of cowpea cultivars with multiple resistances to economically important viruses for onward delivery to farmers to improve productivity for food security.

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