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Occurrence and Distribution of Viruses Associated with Okra and Their Alternative Hosts in Kaduna and Zamfara States, Nigeria.

Zaharaddeen Samaila Gilima*[A], Boniface David Kashina[B], Olalekan Oyeleke Banwo, Mathew Dada Alegbejo[B], Agaat Charles Chindo[B] and Ibrahim Tijjani[A]

[A] Department of Crop protection Faculty of Agriculture Bayero University, P.M.B. 3011, Kano. Nigeria.
[B] Department of Crop Protection Faculty of Agriculture Ahmadu Bello University P.M.B 2240, Zaria, Nigeria.

*Corresponding author; email: zsgilima.cpp@buk.edu.ng

Abstract

One of the major constraints to production of okra (*Abelmoschus esculentus* L.) in Nigeria and in particular in Kaduna and Zamfara States, is the problem of okra mosaic virus and okra leaf curl virus. This study was carried out to provide information on the occurrence and distribution of okra mosaic and okra leaf curl viruses on okra, in Kaduna and Zamfara states, Nigeria. A survey of okra-producing farms was carried out during dry and wet seasons of 2017 cropping season in Kaduna (Zaria, Lere, and Igabi Local Government Areas) and Zamfara (Gusau, Bungudu, and Zurmi LGAs) states. Leaf samples (15) of symptomatic okra plants were collected from each farm in the study area.. The total number of plants and the number of symptomatic plants within each subplot were recorded, and the disease incidence was determined.Double Antibody Sandwich Enzyme-Linked Immunosorbent Assay (DAS-ELISA) kit was used in the detection of Okra Mosaic Virus while Polymerase Chain Reaction (PCR) was employed for the detection Okra Leaf Curl Virus. The results showed that all the okra leaf samples tested for OLCV were negative in this study while OkMV was tested positve in all the samples with a recorded incidence of 20 % and 14 % in Kaduna and Zamfara states respectively, however only 8 out of total weed samples were also tested positive for OKV, but all were tested negative to OLCV.

Keywords: Alternative hosts, disease incidence, okra leaf curl virus, okra mosaic virus

Introduction

Okra (*Abelmoschus esculentus* L.) is one of the most important vegetable crops commonly grown in the tropics, including Nigeria (Schippers, 2002). Okra was cultivated in an estimated area of 3.7 million hectares with an annual production of 3.4 million tonnes worldwide (FAOSTAT, 2017). India, with production estimated at 6.0 metric tonnes on 5.0 million ha (FAOSTAT, 2017), made it the largest producer of okra in the world. However, Nigeria, with production estimated at 2.0 metric tonnes on 1.48 million ha (FAOSTAT, 2017), made it the second largest producer in the world and largest producer of okra in Africa. Furthermore, in Nigeria, North West is among the major okra producing zone in the country, producing 173.2 metric tonnes (Mt). The leading States in okra production in the zone are Kaduna (44.4 Mt), Kebbi (40.9 Mt), and Zamfara (39.4 Mt) (NAERLS et al., 2017). Okra cultivation and production have been widely practiced because of its importance to the economic development. The tender fruits, leaves and shoots are consumed, either in fresh or dried forms (Arapitsas, 2008). Okra consumption among other fruit vegetables is beneficial in moderating blood pressure, fibrinogen concentration and plasma viscosity in Nigerian hypertensives (Adebawoo *et al.* 2007). The fresh pods and leaves of okra are a good source of protein, minerals and vitamins (Lamont, 1999; Arapitsas, 2008).In spite of the fact that, Nigeria is the largest producer of okra in Africa, yields are very low (estimated at 1.2 tonnes/ha) (NAERLS *et al.,* 2017). About 10 to 15 $t.ha^{-1}$ of yield can be obtained under good management (NARP, 1993). Currently, its production is constrained by the prevalence of insect pest and disease attacks. Virus constitutes a major threat to the production of this crop, as it is susceptible to not less than 19 viruses, with *Okra mosaic virus* (OkMV), *Okra leaf curl virus* (OLCV) and *OkraYellow Vein Mosaic Virus* (OYVMV) being the most commonly studied (Swanson and Harrison, 1993; Bhagat et al., 1997; Ali et al., 2005). Among the viruses identified to affect okra in Nigeria, OLCV and OkMV are the most economically important (Lana, 1976; Atiri, 1984; Alegbejo, 2003), they have been

reported as the major diseases of okra in different parts of the country, where an incidence of up to 100% has been reported (Atiri and Ibidapo, 1989; Alegbejo, 1997; Fajinmi and Fajinmi, 2010, Askira, 2012), and the most pressing plant protection problem faced by all okra growers accounting for 30%-80% yield loss. (Lana, 1976, Alebgejo, 1996, Alegbejo et al., 2001).

In the light of the presented reports, a preliminary field observations to gather information from okra farmers in of areas Kaduna and Zamfara States where little or no information has been reported was conducted. Our study revealed the symptoms of these viruses in these areas, indicating the virus presence in other parts of Northern Nigeria. The study was conducted to determine the occurrence and distribution of these viruses associated with okra as well as their alternative hosts in Kaduna and Zamfara States of Nigeria in order extract more knowledge and information on the occurrence and distribution of OLCV and OkMV associated with okra in other parts of Nigeria which is inevitably needed, for effective diagnosis and management of these viruses in order to improve yield.

Materials and Methods

Survey and Sampling of Okra Fields

Surveys were conducted in 2017 dry and wet seasons to determine the occurrence and distribution of Okra mosaic and Okra leaf curl viruses in Kaduna and Zamfara States of Nigeria. Three Local Government Areas (LGAs) per State and three okra farms each per LGA were surveyed for the disease incidence. Selection of the States was based on their high okra production figures (NAERLS et al., 2017), while that of the LGA's and farm locations was based on proximity and information from okra farmers in both States. The survey was spread to capture the areas of low and high production of okra in both states. In Kaduna State, Zaria, Lere, and Igabi Local Government Areas were surveyed while Gusau, Bungudu, and Zurmi LGAs were surveyed in Zamfara State. In each farm, four subplots were demarcated at four ends of the field. Leaf samples (15) of symptomatic and asymptomatic okra plants were collected from each farm. The total number of plants and the number of symptomatic plants within each subplot were recorded. The incidence of the disease was then calculated by dividing the number of positive plants samples by the total number of plants assessed. The corresponding results were converted to percentage incidence by multiplying the value by 100 according to by Chaube and Pundhir (2005) below:

Disease incidence (%) = Number of positive tested samples/Total number of plants examined × 100

During the survey weed species within and around up to five meters away from the okra farm were sampled and randomly collected. All samples were properly labelled and wrapped in polyethylene bags, placed in an ice chest and transported to the Virology Laboratory of the Department of Crop Protection, ABU, Zaria, for diagnosis.The weeds were identified in the herbarium unit in the department of biological science, ABU Zaria.

Laboratory Detection of Okra Mosaic and Okra Leaf Curl Viruses

Double Antibody Sandwich Enzyme-Linked Immunosorbent Assay (DAS- ELISA) kit from Leibniz-Institut DSMZ-Deutsche Sammlung von, Germany was used in the detection of OkMV while Polymerase Chain Reaction (PCR) was employed for the detection of OLCV. This was due to lack of ELISA kit specific for the detection of OLCV. To detect OkMV Specific antibody for Okra mosaic virus (IgG) was diluted in coating buffer at recommended dilution of 1:1000, and 200 µlwas added to each test well of the microtitre plate. The plates were covered and incubated at 37℃ for 2 hours. The plates were washed with PBS-Tween 20 using wash bottle, after soaking it for 3 minutes and the washing was repeated twice. The plates were blotted by tapping upside down on tissue paper. Sap from the samples was extracted in extraction buffer by grinding 1g of tissue in 10 ml of the buffer using sterile mortar and pestle. Two hundred microlitres (200µl) aliquots of the test sample were added to duplicate wells. The plates were covered and incubated overnight at 4℃.The plates were washed 3 times as described above. Two hundred microliters (200µl) enzyme conjugate (IgG-AP diluted in conjugate buffer at the recommended ratio of 1:1000) was added to each well. The plates were covered and incubated at 37℃ for 3 hrs. The plates were washed 3 times as described above. Two hundred microlitres (200µl) aliquots of the freshly prepared substrate (1 $mg.ml^{-1}$ para-nitrophenyl-phosphate in substrate buffer with a dilution ratio of 1:1) were added to each well. The plates were covered and incubated at 37°C for 60 mins, for a colour change. Results were assessed visually and the absorbance values were measured using ELISA reader (Biochrom EZ Read 400) at A405 nm (Clark and Adams, 1977). Absorbance values at least twice that of the negative control (check) were rated positive as reported by Kumar (2009).

The PCR was performed in 25 µl total volume using 12.5 µl of 2x master mix (Quick-load Taq), 1 µl each of both forward and reverse primers, 1µl DNA

template, and 9.5µl of nuclease free sterile distilled water. A pair of *Okra leaf curl virus* specific primer (Sohrab, *et al.* 2013), corresponding to (Forward; 5' -TTATGTCGAAGCGAGCTGCC 3'; and Reverse 5' -TTTCAATTCGTTACAGAGTCATA 3' was used to amplify the DNA Coat Protein (CP) gene. The amplification cycling conditions consisted of initial denaturation at 94℃ for 4 min, followed by 30 cycles of denaturation at 94℃ for 1 min, annealing at 55℃ for 1 min, and extension at 72℃ for 1 min, with a final extension of 72℃ for 10 min. PCR products were separated on 1% agarose gels by electrophoresis, visualized using a UV transilluminator for the band sizes. The band size of about 800 base pair (bp) was checked for the presence of *Okra leaf curl virus*.

Results

Farm size, location of sample collection, and symptoms of viral diseases observed on okra in Kaduna and Zamfara states are described in Table 1. The results showed that most of the okra growers in the two states were small scale farmers with an overall average farm size of 0.029 ha. The average farm size for Zamfara State (0.026 ha) was smaller than that of Kaduna State (0.032 ha). However,the different symptoms observed on the field from the two states were mosaic, leaf curl, vein enation,mottling, distortion, plant stunting, chlorosis and necrosis). Mosaic, leaf curl, yellowing, and vein chlorosis, mottling, were very much pronounced on most of the samples.These symptoms were expected to be caused by OkMV and OLCV.

Table 2 showed the incidence of *Okra mosaic virus* infecting okra in all the six Local Government Area (LGA) of both Kaduna and Zamfara State. Igabi LGA, ELISA results showed that out of the forty five samples tested three were positive for OkMV. Birnin Yero had the highest incidence of 13.3 % followed by Rigachukun with 6.7 %, while no incidence was detected in Zangon Aya. In LGA, ten out of forty five samples tested positive to OkMV. Gadan Agwaro recorded the highest incidence of 33.3 % followed by Dakace with 20 % and Galma with 13.3 %. In Lere LGA, ELISA result showed that out of forty five samples tested fourteen were positive to OkMV. Anguwar Bawa recorded the highest incidence of 40% followed by Anguwar Makama with 33.3% and Kauran Dangambo with 20%. In Kaduna state,

In Gusau LGA, ELISA result showed that out of the forty five samples tested nine were positive. Birnin Ruwa had the highest incidence with 26.6% followed by Wanke with 20% and Damba with 13.3%. In Zurmi LGA, four samples were positive out of forty five samples tested. Birnin Tsaba exhibited the highest incidence of 13.3% followed by Kwashebawa and Moriki with 6.7%. In Bungudu LGA, six out of forty five samples tested positive. Bela Rawiya had the highest incidence of 40% followed by Tazame with 13.3% while no incidence was detected Mahuta.

Table 4 showed the weed hosts of *Okra mosaic virus* infecting okra in Kaduna and Zamfara states.Most of the weed samples collected did not exhibited any virus disease symptoms on the field except for few. Weeds samples collected included common weeds belonging to twelve different plant families in Kaduna and Zamfara States respectively. Out of thirty weed samples, only eight from the wet season tested positive to OkMV (Table 4).

Figure 3 A and 3 B showed the incidence of Okra mosaic virus infecting okra in Kaduna and Zamfara states. In Kaduna state, Lere LGA had the highest incidence recorded with 31% followed by Zaria LGA with 22.2%. The lowest incidence was recorded in Igabi LGA with 6.6% as shown in Figure 3A. However, in Zamfara State, Gusau LGA had the highest incidence recorded with 20% followed by Bungudu LGA with 13.3%. The lowest incidence was recorded in Zurmi LGA with 8.8% (Figure 3B). Moreover, the total incidence recorded in Kaduna state was 20% and in Zamfara state was 14% as shown in Figure 4. Hence, Kaduna state recorded higher incidence of *Okra mosaic virus* than Zamfara state.

Discussion

This study reported the incidence, distribution and weed host of *Okra mosaic virus* in Kaduna and Zamfara states. The symptoms observed on the leaves samples during samples collections which include mosaic, leaf curl, vein enation,mottling, distortion, plant stunting, chlorosis and necrosishave been reported to be associated with the virus (Brunt et al., 1996; Krishnareddy et al., 2003; Alegbejo et al. 2008). Symptom expression is influenced by several factors including: virus species/strain (Harrison et al., 1997; Owor, 2003), host response (Otim-Nape, 1993; Byabakama et al., 1997; Otim-Nape et al*.,* 1998; Sserubombwe et al., 2001), plant age at infection (Otim-Nape, 1987; Fargette et al., 1988), temperature (Gibson, 1994) and soil fertility (Spittel and Van Huis, 2000; Seruwagi et al*.,* 2003).

In this study, symptom expression could be primarily attributed to the host response with less contribution from other factors. Some of the samples tested had virus-like symptoms, but no virus was detected using the serological method employed in this research.

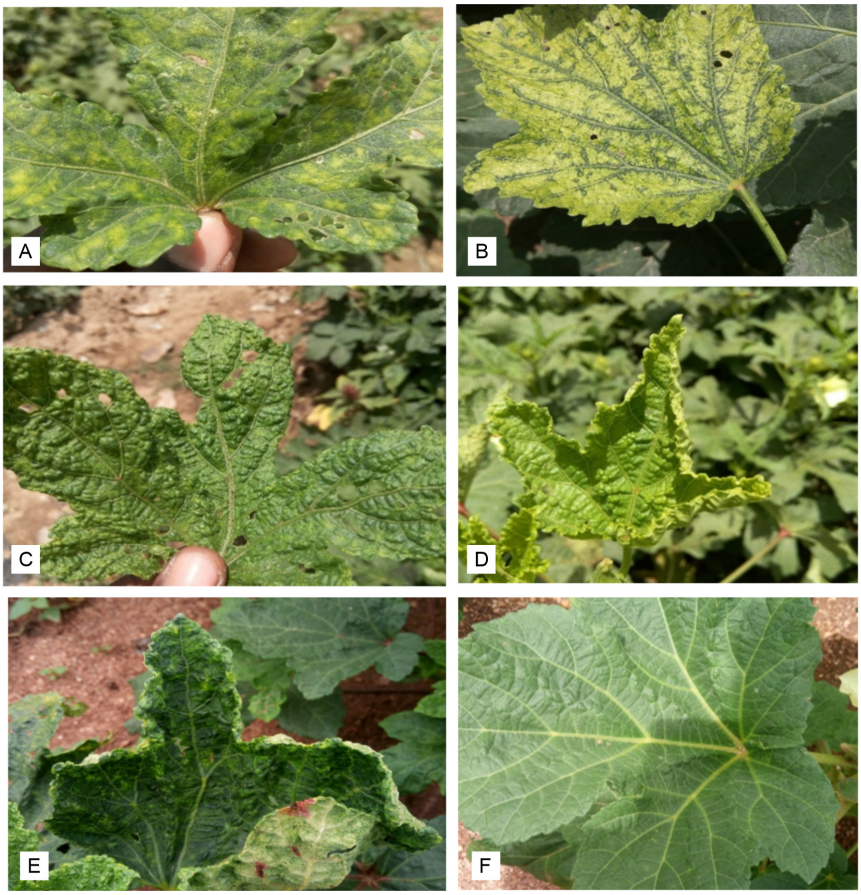

Figure 1. Okra leaves exhibiting mosaic (A), chlorosis/vein banding (B), enation /leaf crinkling (C) and leaf curl and mosaic (D and E) symptoms compared with healthy leaf (F).

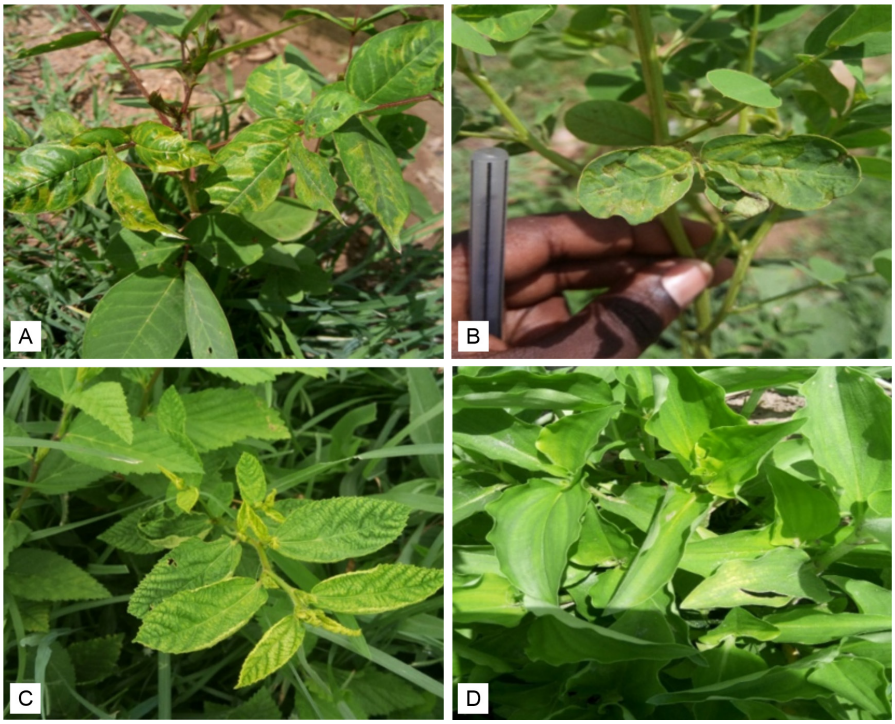

Figure 2. Weed samples exhibiting mosaic symptoms on *Senna occidentalis* (A), enation /leaf crinkling symptoms on *Senna obtusifolia* (B), leaf curl and vein chlorosis symptoms on *Sida acuta* (C), and *Commelina benghalensis* showing no visible symptom (D).

Table 1. Farm size, location, and symptoms of viral diseases observed on okra in Kaduna and Zamfara states

State	LGA	Location	Area (m^2)	Number of plants obseved	Observed symptoms
Kaduna	Zaria	Galma	402	104	LC,St, M, Mt
		Dakace	152	95	M, Mt, C
		Gidan agwaro	365	171	St, M,VE
	Igabi	Zangon Aya	398	233	M, C, St
		Birnin yero	325	167	LC, M, VE
		Rigachukun	168	53	M, Mt, C
	Lere	Unguwar bawa	625	356	M, St, LC, C
		Anguwar makama	214	105	M, St, Mt
		Kauran dangambo	242	153	LC, M, Mt,St
Zamfara	Gusau	Danba	228	108	M, LC, VE
		Birnin ruwa	914	315	LC, Mt, M
		Wanke	425	238	M, C, N
	Zurmi	Kwashebawa	118	92	Mt,C,VE, LC
		Birnin tsaba	167	106	LC, M, Mt
		Moriki	231	113	M, Mt, C
	Bungudu	Bela rawaiya	178	90	LC, M, Mt
		Tazame	192	130	M, Mt, St
		Mahuta	297	168	C, Mt, N

Note: LGA=Local Government Area,M = mosaic, LC = leaf curl, VE = vein enation,Mt = mottling, St = stunting, C = chlorosis and N = necrosis

Table 2. Incidence of *Okra mosaic virus* in the six Local Government Area of Kaduna and Zamfara states

LGA	Location	% Virus disease incidence (Number of infected plants out of 15)
Igabi	Zangon aya	0 (0/15)
	Birnin yero	13.3 (2/15)
	Rigachukum	6.7 (1/15)
Zaria	Galma	13.3 (2/15)
	Dakace	20 (3/15)
	Gadan agwaro	33.3 (5/15)
Lere	Anguwar bawa	40 (6/15)
	Anguwar makama	33.3 (5/15)
	Kauran dangambo	20 (3/15)
Gusau	Damba	13.3(2/15)
	Birnin ruwa	26.6 (4/15)
	Wanke	20 (3/15)
Zurmi	Kwashebawa	6.7 (1/15)
	Birnin tsaba	13.3 (2/15)
	Moriki	6.7 (1/15)
Bungudu	Tazame	13.3 (2/15)
	Mahuta	0 (0/15)
	Bela rawaiya	13.3 (4/15)

Note: LGA = Local Government Area

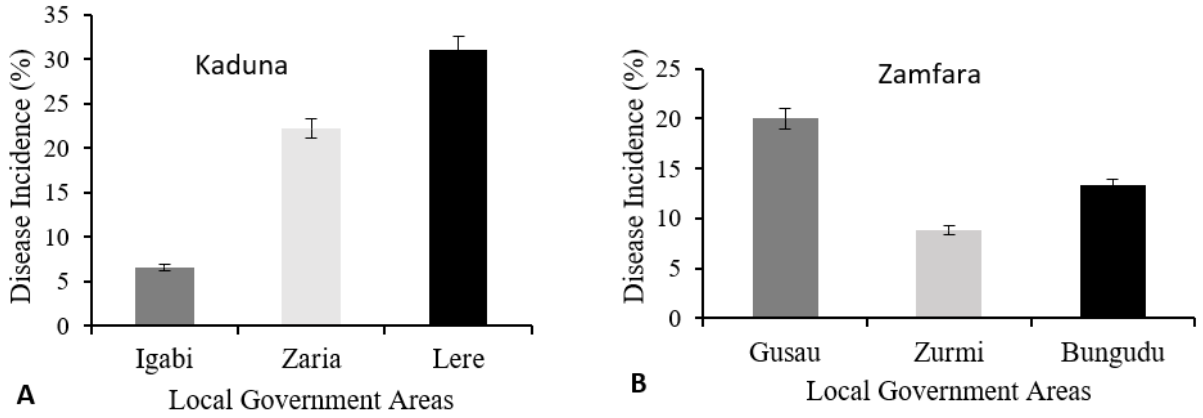

Figure 3. Incidence of Okra mosaic virus in Kaduna state (A) and Zamfara state (B)

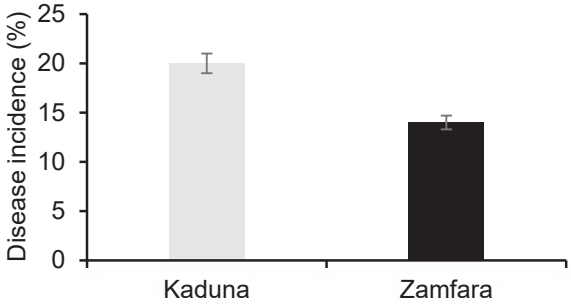

Figure 4. Total disease incidence in the state of Kaduna and Zamfara.

This might have been caused by other viruses with similar symptoms that their antiserums have not been used. As it has been reported, okra is susceptible to not less than 19 viruses (Swanson and Harrison, 1993).

From the results of this work, all the leaf and weed samples tested against OLCV virus using PCR were negative, even though the symptoms were observed on the okra infected leaves in the field, like it was first reported in Ibadan (Atiri and Ibidapo, 1989), in Lake Alau of Borno state, Nigeria (Askira, 2012) and in Ghana (Asare-Bediako et al., 2014b) who reported that more than 50% of the farms surveyed were seriously devastated by the virus. Although in all the reports above molecular test were not conducted to confirm the incidence detected on the field.

However, the result showed that *okra mosaic virus* occurred in all the survey areas of all the three local government areas except Zangon Aya in Igabi LGA, Kaduna State and Mahuta in Bungudu LGA, Zamfara state. This finding could be attributed to low virus titre in the plant samples tested in the areas or it could be attributed to non-weedy nature of the farms surveyed in this locations as reported by George (2003). Another reason could be the effect of the mixed cropping pattern practiced in the all the farms in these two locations, reducing the risk of flea beetles infestation, as the virus is transmitted by the insect in a non-persistent manner. This is similar with the findings of Ahmed et al. (2006), Pitan and Olatunde (2006) reported that, intercropping okra with crops like cotton, tomato, cowpea, and groundnut serves as control for okra flea beetles (*Podagrica* spp.). As for the samples from other locations tested positive, disease with similar symptoms have been reported in the South West of Nigeria (Fajinmi and Fajinmi, 2010a, 2010b) and in Samaru, Shika, Bomo areas in northern Nigeria (Alegbejo et al., 2008). The incidence of the virus was recorded to be higher in Lere LGA with 31% followed by Zaria LGA with 22.2% all in Kaduna State, and then Gusau with 20% in Zamfara state. This agrees with the findings of Atiri (1984) who reported an incidence of up to 100% before harvest in Ibadan, Nigeria. OkMV incidence of up to 30 to 89% has also been reported in Tanzania (Nduguru and Rajabu, 2004). The incidence of the OkMV could have been due to weedy nature in and around the okra farms which serves as reservoir for both the vector and the virus. Weed species have been reported to be important source of OkMV (Atiri, 1984; Alegbejo, 2001, 2000; Alegbejo et al. 2008).

A wide range of weeds were observed in both okra fields and surrounding farms. Studies by Atiri (1984) and Alegbejo (2001, 2008) revealed that the endemic nature of OkMV in Nigeria could be due to weed

Table 3. Weed species tested against Antisera of *Okra mosaic virus* infecting okra in Kaduna and Zamfara State of Nigeria

Weed species	Okra mosaic virus	
	Wet season	Dry season
Alternanthera sessilis DC. (Linn)	+	NF
Tridax procumbens Linn.	-	NF
Synedrella nodiflora Gaertn.	-	NF
Heliotropium indicum L. indian	+	NF
Commelina erecta L.	-	NF
Cyperus esculentus Linn.	-	NF
Cyperus iria Linn.	+	NF
Rhynchospora corymbosa (Linn.) Britt.	-	NF
Kyllinga bulbosa Beauv.	-	NF
Sida acuta Burm.	-	NF
Senna occidentalis (L.) Link.	+	NF
Senna obtusifolia (L.) Irwin	+	NF
Urena lobata Linn.	-	NF
Digitaria horizontalis Willd	-	NF
Elusine indica Gaertn.	-	NF
Talinum triangulare (jacq) willd.	-	-
Amaranthus spinosus Linn.	+	-
Hyptis lanceolate Poir.	+	-
Axonopus compressus (Sw.) P. Beauv	NF	-
Commelina benghalensis L.	NF	-
Cynodon dactylon (Linn.) Pers.	NF	-
Eclipta alba (L.) Hassk.	NF	-
Portulaca quadrifida Linn.	NF	-
Spermacoce verticillata Linn.	+	-
Spermacoce ocymoides Burn. F.	NF	-
Vernonia capensis (Houtt.) Druce	NF	-
Total (+)	8(30)	

Note = Not detected, + = Detected, NF= Not found.

hosts of the virus found in and around Okra fields and serves as important source of inocula. Also, Fajinmi and Fajinmi (2010b) reported the implication of weed host as reservoir of OkMV.This part of the studies established that out of the thirty (30) weed samples collected in the two states, eight weed samples *Alternanthera sessilis DC.,Amaranthus spinosis* Linn*., Senna occidentalis* (L) Link., *Senna obtusfolia* (L.) Irwin and barneby*,Sida acuta* Burm, *Heliotropium indicum* L. Indian*, Cyperus iria* Linn., and *Spermacoce verticillata* Linn., belonging to six families tested positive to OkMV in Kaduna and Zamfara states. Our result are in line with the findings of Atiri and Ekpo (1982), Atiri (1984), Alegbejo (2001, 2008) and Fajimi and Fajimi (2010b), that OkMV has a wide host range of weed species belonging to the families Malvaceae, Solanaceae, Commelinaceae, Amaranthaceae and Euphobiacaea, so these families serve as virus reservoirs for OkMV.

Conclusion

The prevalence of *Okra mosaic virus* (OkMV) in Kaduna and Zamfara States was demonstrated in this report with an incidence of 19.9% and 14% respectively, this representing the first report of OkMV in Zamfara State, and other parts of Kaduna State studied, it was also detected in eight weeds samples from six families during wet season only, however,

Okra leaf curl virus (OLCV) was not detected in all the samples collected in both Kaduna and Zamfara states despite the presence of visible symptom, owing to many reason which are subject to further investigations on the virus.

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