DETECTION AND DISTRIBUTION OF VIRUSES INFECTING SWEET POTATO (*Ipomoea batatas* L. LAM.) AND THEIR ASSOCIATED VECTORS IN KEBBI AND KATSINA STATES, NIGERIA

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SUMMARY

Sweet potato is a food security crop because of its ability to withstand adverse climatic conditions and provides sustainable food when other crops fail. This security, however, is being threaten by viral diseases. Surveys were conducted for viruses infecting sweet potato in 2020 rainy and 2021 dry seasons in Kebbi and Katsina States. Samples were collected from sweet potato plants and tested for Sweet potato chlorotic fleck virus (SPCFV), Sweet potato mild mottle virus (SPMMV) and Sweet potato virus 2 (SPV2) using Double Antibody Sandwich Enzyme-Linked Immunosorbent Assay (DAS-ELISA). Whitefly and aphid populations were assessed. Results revealed that, the three viruses infected sweet potato in the study areas. Kebbi had higher incidence of SPCFV (17.78%), SPMMV (11.11%), and SPV2 (25.56%) in the dry season, while Katsina had higher incidence of SPCFV (10.00%) in the rainy season. Mean disease incidence in Kebbi was significantly (P = 0.05) higher than in Katsina. Mean symptom severity was not significant (P = 0.05) in the two states and for the two seasons. Aphid was not observed in the rainy season in both states. In the dry season, the mean aphid population per leaf was not significant (P = 0.05) in both states. The mean whitefly population was significantly (P = 0.05) higher in Kebbi (8.86) than in Katsina (6.34) in the dry season, but in the rainy season it was not significant in both states. There was no significant relationship between aphid population and SPV2 incidence, between whitefly population and SPMMV incidence in both states and seasons. SPCFV, SPMMV, and SPV2 occurred in the study areas. Farmers need to be trained on symptom identification and the importance of using only disease-free vines as planting materials.

Keywords: Aphid, DAS-ELISA, SPCFV, SPMMV, SPV2, whitefly

SWEET POTATO, *Ipomoea batatas* L. (Lam.), is an important economic food crop in sub-Saharan Africa (SSA). In terms of annual production, sweet potato ranks as the fifth most important food crop in the tropics and the seventh in the world food production after wheat, rice, maize, potato, barley, and cassava (FAOSTAT, 2019). Sweet potato fulfills several basic roles in the global food system, all of which have fundamental implications for meeting food requirements, reducing poverty, and increasing food security (El Sheikha and Ray, 2017). The storage root of sweet potato is very rich in carbohydrate and its vine tips are used as vegetables in some countries and the entire leaves serve as feed for livestock (Tavva and Nedunchezhiyan, 2012). It is a good source of protein, lipid, calcium and carotene besides carbohydrates content (Laurie *et al*., 2018).

Over thirty viruses have been reported to infect sweet potatoes worldwide (Barkessa, 2018). The major economic important sweet potato viruses are: *Sweet potato feathery mottle virus* (SPFMV), *Sweet potato chlorotic stunt virus*(SPCSV), *Sweet potato virus G* (SPVG), *Sweet potato mild mottle virus* (SPMMV), *Sweet potato chlorotic fleck virus* (SPCFV), *Sweet potato latent virus* (SPLV), *Cucumber mosaic virus* (CMV), *Sweet potato caulimo-like viru*s (SPCaLV) *and Sweet*

potato leaf curl virus (SPLCV) (Barkessa, 2018). Before developing any disease management measure, it is important to know the actual pathogen causing the problem, its status and distribution in the affected area. Identification and detection to establish the identity of the viruses infecting sweet potatoes is an important foundation for their management. Knowing the correct identity of a virus aids in selecting appropriate resistance sources, developing vector control plans, and selecting other appropriate tools such as planting dates, sanitation plans, and eradication protocols (Ownley and Trigiano, 2016). There is no record of work on the identification and distribution of viruses infecting sweet potatoes in Kebbi and Katsina States of Nigeria. This study was therefore conducted to provide information on the identity and distribution of viruses infecting sweet potatoes and their associated insect vectors in Kebbi and Katsina States, Nigeria.

MATERIALS AND METHODS

Field Survey

Field surveys were conducted in August 2020 rainy and January 2021 dry seasons. Three sweet potato-growing Local Government Areas (LGAs) each were selected from each of the two States. In each LGA, three sweet potato fields were sampled. In each field, 30 sweet potato plants were randomly sampled along two diagonals (X) and examined for symptoms of viruses infecting sweet potatoes, disease incidence, symptom severity, and vector abundance (Sseruwagi *et al*., 2004). Sweet potato variety, age, cropping system, crops grown on neighboring fields, field size and weather conditions (Temperature and Relative humidity) during the time of the survey and other relevant epidemiological information were collected in a survey data sheet. Geographical Positioning System (GPS) co-ordinates (latitude, longitude, and elevation) were also collected from each field to produce the disease distribution map.

Sample collection and preservation

Symptomatic and asymptomatic leaf samples were collected, and samples were collected during the rainy and dry seasons. The samples collected were preserved separately in bottles containing calcium chloride, and taken to Virology Laboratory, Department of Crop Protection, Ahmadu Bello University, (ABU) Zaria for analysis.

Disease incidence and symptom severity

Disease observation and leaf sampling were carried out randomly along two diagonals (X) on 30 sweet potato plants aged 2-3 months. Disease incidence was determined based on the appearance of the symptoms on plants sampled. Disease incidence of each field was calculated as the percentage (%) of visually diseased plants from the total plants assessed in the field using the formula of Hidayat *et al*. (2020).

Disease Incidence (%) = $\frac{n \times 100}{N}$

Where: n = number of symptomatic plants and N = sum of all plants assessed.

Symptom severity of each diseased plant sampled in a field was scored based on the percentage of the leaf surface showing virus disease symptoms as described before on cassava leaves by Eni *et al.* (2020) with modification using the scale of 1 to 5 shown below:

Score	Description
1	Asymptomatic plant
2	Plant with 25% of leaf surface showing mild mottling or vein clearing
3	Infected plant with 50% of leaf surface exhibiting moderate mottling, vein clearing, chlorosis
4	Infected plant with 75% of leaf surface exhibiting severe mottling, vein clearing, leaf distortion and general reduction of leaf size
5	Infected plant with more than 75% of leaf surface exhibiting very severe mottling, vein clearing, leaf distortion, and in most cases stunted growth.

Mean symptom severity of each field was calculated and used to compute the mean symptom severity of each LGA and State.

Virus incidence

Virus incidence (%) was calculated using the formula of Chaube and Pundhir (2005). This was achieved after laboratory analysis of the leaf samples.

$$\text{Virus Incidence (\%)} = \frac{Number\ of\ positive\ samples/field}{Total\ number\ of\ samples\ examined/field} \times 100$$

Estimation of Whitefly and Aphid population

Evaluation of aphid and whitefly population in a field was achieved by direct counting of adults on five youngest apical leaves of the shoots of each of the 30 plants (Ndunguru *et al*., 2009). Whitefly was collected using aspirator while aphid was collected using camel hairbrush. Both whitefly and aphid collected were preserved separately in Eppendorf tubes containing 70% ethanol which were taken to Insect Museum of Department of Crop Protection, Faculty of Agriculture, Ahmadu Bello University, Zaria for identification.

Enzyme-linked immunosorbent assay (ELISA) procedure

Leaf samples were ground in a sterilized mortar and pestle with sample extraction buffer as supplied by DSMZ, Germany at a ratio of 1:10 (tissue weight in g: buffer volume in ml).

Purified coating antibody (IgG) was diluted in coating buffer at recommended dilution ratio of 1:1000. Two hundred microlitres (200 µl) of this mixture were added to each well of a microtiter plate and incubated at 37^0C for 3 hours. After incubation, the content was decanted into a sink using a quick flip motion and Phosphate Buffered Saline-Tween 20 (PBS-T) was used to wash the plate three times after soaking for a few minutes. Plate was blotted by tapping upside down on a towel to remove droplets of buffer and bubbles. Two hundred microlitres (200 µl) of the test sample extracted in sample extraction buffer (1:10) was added to duplicate wells and incubated overnight at 4^0C. PBS-T was used to wash the plate thrice as described earlier.

Another 200 µl of IgG-AP- enzyme conjugate diluted in conjugate buffer (1:1000) was added to each well, incubated at 37^0C for 3 hours and washed with PBS-T as done earlier. Finally, 200 µl aliquots of freshly prepared subtract (i.e., p-nitrophenyl phosphate) which was dissolved in subtract buffer at ratio 1:1, was added to each well and incubated at 37^0C for 60 minutes. The plates were assessed visually against the controls. The wells that were positive developed yellow color as the positive control while those that were negative remained colorless. Thereafter, spectrophotometer measurement of absorbance at 405 nm was taken using the ELISA

spectrophotometer (BIO RAD Microplate Reader, iMark). An absorbance value twice the values of negative control was rated virus as positive (Gibson and Kreuze, 2015).

Data analysis

Data generated were analyzed using IBMSPSS statistics software version 20. Descriptive statistics tools such as means, percentages, and standard deviation were used to determine significance of the results (Chaube and Pundhir, 2005).

RESULTS

Symptoms observed in the fields

The predominant symptoms characteristics of virus infection observed were mottling, vein clearing, purpling of the leaves, stunted growth, and inward leaf curling. Mottling was the most common symptom observed in almost all the infected fields surveyed in the rainy season, followed by vein clearing and the stunted growth; while in the dry season survey, vein clearing was the most common symptom observed, followed by inward leaf curling, and stunted growth (Plates I – VI).

Plate I: Symptomless

Plate II: Mottling on

Plate III: Leaf curling

Plate IV: Leaf purpling

Plate V: Vein clearing

Plate VI: Stunted

Disease incidence and symptom severity

Mean disease incidence was significantly higher (p = 0.05) in Kebbi (65.19%) and (62.96%) than in Katsina (50.38%) and (54.82%) in rainy and dry seasons, respectively. In Kebbi State, Aliero LGA had the highest disease incidence (72.23%), followed by Jega LGA (67.80%); while the least disease incidence was observed in Mayama LGA (55.53%) (Table 1). In the dry season, Aliero and Jega exhibited high disease incidence (68.89%) and (64.44%) respectively, while Mayama showed 55.55%. In Katsina State, Funtua LGA had the highest disease incidence (64.44%), followed by Bakori LGA (50.01%); while Danja LGA showed the least disease incidence

(36.68%) in the rainy season. In the dry season, Bakori had the highest disease incidence (66.67%); while Funtua and Danja LGAs had same disease incidence (48.89%) (Table 2). Mean symptom severity per plant sampled was statistically mild in all the LGAs in both seasons. Of the leaves of a diseased plant sampled, 25% showed mild mottling or vein clearing.

Table 1: Mean disease incidence and symptom severity during 2020 rainy and 2021 dry seasons in Kebbi State

LGA	No. of Fields	2020 Rainy season		2021 Dry season	
		Incidence (%)	Severity	Incidence (%)	Severity
Aliero	3	72.23	2.20	68.89	2.00
Jega	3	67.80	2.02	64.44	2.00
Mayama	3	55.53	1.86	55.55	1.81
Overall mean	9	65.19	2.03	62.96	1.94
Std. Deviation		7.49	0.15	5.89	0.19

LGA = Local Government Area; Std. = standard; No. = Number

Table 2: Mean disease incidence and symptom severity during 2020 and 2021 seasons in Katsina State

LGA	No. of Fields	2020 Rainy season		2021 Dry season	
		Incidence (%)	Severity	Incidence (%)	Severity
Bakori	3	50.00	1.77	66.67	2.30
Danja	3	36.67	1.57	48.89	1.83
Funtua	3	64.44	1.87	48.89	1.80
Overall mean	9	50.37	1.74	54.82	1.98
Std. Deviation		12.03	0.13	8.89	0.24

LGA = Local Government Area; Std. = standard; No. = Number

Incidence of viruses infecting Sweet potato in the study areas

The ELISA results obtained showed that *Sweet potato mild mottle virus* (SPMMV), *Sweet potato chlorotic fleck virus* (SPCFV), and *Sweet potato virus 2* (SPV2) occurred in both States surveyed but, with significant ($p = 0.05$) variation in distribution.

In Kebbi State, SPCFV was detected only in Aliero LGA with incidence of 13.33%. SPMMV occurred in Aliero 6.67% and Jega 3.33%. SPV2 occurred in the three LGAs of Kebbi State, Jega LGA had highest virus incidence of 6.67% while, Aliero and Mayama LGAs had 3.33% (Table 3) in the rainy season. In Katsina State, SPCFV was detected in two LGAs. Bakori LGA had 26.67% and Funtua had 3.33% virus incidence. SPMMV was also detected in the same two LGAs with Funtua had 6.67% and Bakori 3.33% virus incidence. SPV2 was not detected.

In the dry season, SPCFV was not detected in Bakori LGA, while Danja and Funtua LGAs exhibited the same SPCFV mean (6.67%) incidence; SPMMV was detected only in Bakori LGA (6.67%). SPV2 was detected in Danja (26.67%) and Funtua (10.00%) (Table 4). In Kebbi State, all the three viruses were detected in the three LGAs (Table 3).

Table 3: Mean incidence of the three viruses detected in Kebbi State in the 2020 rainy and 2021 dry seasons.

Local Government Area	No. of Fields	Mean virus incidence (%)					
		Rainy season			Dry season		
		SPCFV	SPMMV	SPV2	SPCFV	SPMMV	SPV2
Aliero	3	13.33	6.67	3.33	20.00	13.33	3.33
Jega	3	0.00	3.33	6.67	23.33	6.67	16.67
Mayama	3	0.00	0.00	3.33	10.00	13.33	56.67
Overall mean	9	4.44	3.33	4.44	17.78	11.11	25.56
Std. Dev.		6.67	2.89	1.67	6.01	3.33	24.04

Std. = standard, No. = Number, SPCFV = *Sweet potato chlorotic fleck virus*, SPMMV = *Sweet potato mild mottle virus*, SPV2 = *Sweet potato virus 2*

Table 4: Mean incidence of the three viruses detected in Katsina State in the 2020 rainy and 2021 dry seasons.

Local Government Area	No. of Fields	Mean virus incidence (%)					
		Rainy Season			Dry Season		
		SPCFV	SPMMV	SPV2	SPCFV	SPMMV	SPV2
Bakori	3	26.67	3.33	0.00	0.00	6.67	0.00
Danja	3	0.00	6.67	0.00	6.67	0.00	26.67
Funtua	3	3.33	0.00	0.00	6.67	0.00	10.00
Overall mean	9	10.00	3.33	0.00	4.45	2.22	12.22
Std. D.		12.59	2.88	0.00	3.34	3.34	11.67

Std. = standard, No. = Number, SPCFV = *Sweet potato chlorotic fleck virus*, SPMMV = *Sweet potato mild mottle virus*, SPV2 = *Sweet potato virus 2*

Distribution of the viruses infecting sweet potatoes in Kebbi and Katsina States

The distribution of the viruses infecting sweet potatoes in Kebbi and Katsina States during both seasons varied from one LGA to another. SPMMV was the most prevalent virus infecting sweet potato in the study areas and distributed in four LGAs of both States. SPCFV was found in Aliero LGA of Kebbi State, Bakori and Funtua LGAs of Katsina State. SPV2 was detected in Aliero, Jega, and Mayama LGAs. For the dry season, SPCFV, SPMMV and SPV2 were distributed in Aliero, Jega, and Mayama LGAs of Kebbi State. In Katsina State, SPCFV and SPV2 were found in Danja and Funtua LGAs. SPMMV was present in only Bakori LGA of Katsina State (Figs. 1 and 2).

Whitefly and Aphid population

Statistical analysis revealed no significant (p >0.05) difference in whitefly abundance between Kebbi and Katsina States in the rainy season. In Kebbi, Jega LGA had the highest mean whitefly population which averaged 2 adult whiteflies per leaf. In the dry season, Jega LGA had the highest mean 12 adult whiteflies per leaf followed by Mayama 8 adult whiteflies per leaf and Aliero 7

adult whiteflies per leaf LGAs. In Katsina State, the highest mean whitefly population was 2 adult whiteflies per. In the dry season, the highest mean 8 was recorded in Bakori LGA. Statistically, aphid population was significantly lower as compared with whitefly population in both States. Highest mean aphid population was observed in Bakori LGA of Katsina State which averaged 4 aphids per leaf, followed by Mayama LGA of Kebbi State which average 2 aphids per leaf (Tables 5 and 6).

Table 5: Mean Whitefly and Aphid population during 2020 rainy and 2021 dry seasons in Kebbi State, Nigeria.

Local Government Area	No. of Fields	2020 Rainy season		2021 Dry season	
		Whiteflies	Aphids	Whiteflies	Aphids
Aliero	3	1	0	7	2
Jega	3	2	0	12	2
Mayama	3	1	0	8	2
Overall mean	9	1	0	9	2
Std. Deviation		0.49	0.00	2.23	0.07

Std. = standard, No. = Number

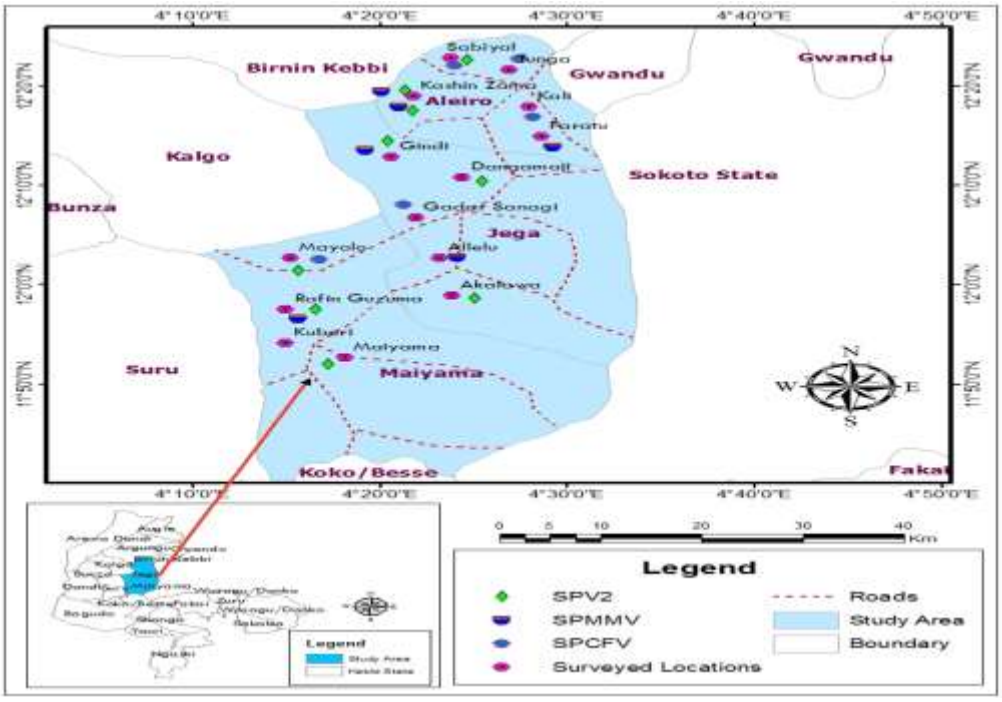

Figure 1: Distribution viruses infecting sweet potato in Kebbi state, Nigeria in both Seasons

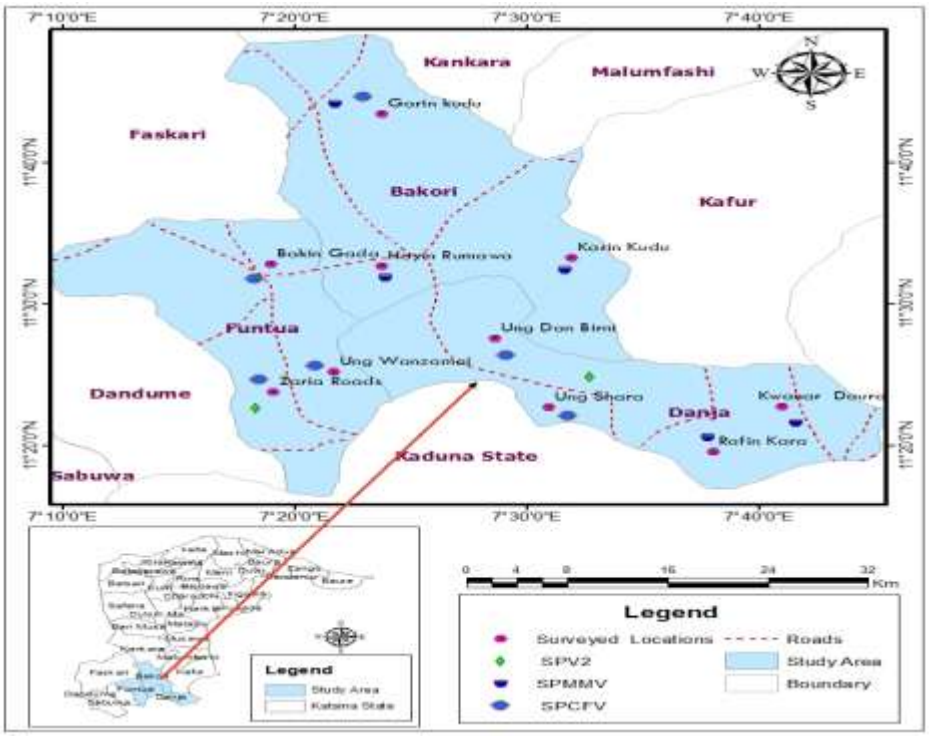

Table 6: Mean Whitefly and Aphid population during 2020 rainy and 2021 dry seasons in Katsina State, Nigeria

Local government	No. of Fields	2020 Rainy season		2021 Dry season	
		Whiteflies	Aphids	Whiteflies	Aphids
Bakori	3	1	0	8	4
Danja	3	2	0	5	2
Funtua	3	2	0	7	1
Overall mean	9	2	0	7	2
Std. Deviation		0.46	0.00	1.39	1.09

Std. = standard, No. = Number

DISCUSSION

Symptoms induced by viruses infecting sweet potatoes were observed in the study areas. Mottling, vein clearing, and purpling were observed on leaf samples from which SPCFV, SPMMV, and SPV2 were tested. Other virus-like symptoms such as stunted growth, and inward leaf curling observed on some samples which tested negative against SPCFV, SPMMV, and SPV2 might be because of other viruses infecting the crop which were not tested in this study or phytoplasmas, or due to other abiotic factors such as nutrient deficiency (Barkessa, 2018). In this study, mottling and vein clearing were the predominant symptoms observed in farmers' fields. Similar symptoms were reported in Nigeria and other African countries (Ndunguru *et al*., 2009; Sivparsad and Gubba, 2013; Kwak *et al*., 2014; Mhammed, 2018). Wasswa (2012) reported leaf curling and chlorosis as

predominant symptoms in farmers' fields in Uganda. Sivparsad and Gubba, (2013) reported leaf deformation, leaf curling, and chlorosis in KwaZulu-Natal province, South Africa. The serological test conducted confirmed that the symptoms observed were induced by viruses infecting sweet potatoes.

Higher mean disease incidence was recorded in Aliero and Jega LGAs of Kebbi State in both seasons and lower mean disease incidence in Danja LGA of Katsina State. The common practice of using vines from previous harvest that might be infected as planting material for the following season among farmers as observed in the study areas might have been a factor for the higher disease incidence in the two LGAs of Kebbi State. Poor field sanitation observed in Kebbi might also contributed to a greater extend the higher incidence in the State. This is in line with the work of Gibson and Kreuze (2015) who reported that the main plant and weeds serve as reservoir for viruses infecting sweet potatoes. Mohammed (2018) reported higher disease incidence in Giwa LGA of Kaduna State and attributed it to weedy farms. Sivparsad and Gubba (2013) also reported higher disease incidence in KwaZulu-Natal province, South Africa. Kim *et al*. (2017) also reported high disease incidence in Korea which they attributed to exchange of sweet potato vines that might be infected among farmers in their study areas. This implies that farmers in the study areas are either unaware of the need for use of virus-free planting materials or have limited access to virus-free planting materials.

Findings from this study showed that symptom severity of sweet potato virus disease was not significant and averaged 2 (that is plant with 25% of leaf showing mild mottling or vein clearing) in both states and seasons. This is contrary to the work of Mohammed (2018) who reported severe symptoms of sweet potato virus disease in Kaduna State, (Ndunguru *et al*., 200914), also reported severe symptom of sweet potato virus disease in Uganda. Low virus concentration in some infected cultivars of sweet potatoes as earlier reported by Wasswa (2012) might to a greater extend be a factor for the mild symptoms of sweet potato virus disease observed in almost all the infected fields visited during the surveys.

Serological results showed the presence of SPCFV, SPMMV and SPV2 in Kebbi and Katsina States of Nigeria. This is the first report of SPCFV, SPMMV, and SPV2 in Kebbi and Katsina States of Nigeria and the first report of SPCFV and SPMMV in northern Nigeria. Based on percentage virus incidence, SPV2 was the predominant virus infecting sweet potatoes in the study areas and the higher virus incidence was recorded in the dry season. The higher incidence of SPV2 observed in the dry season in both States compared to the rainy season can probably be attributed to its dependence on transmission by aphid (*Myzus persicae*) which was not observed in the rainy season survey in both States and was observed in the dry season. A previous work reported *Sweet potato chlorotic stunt virus* (SPCSV) as the most prevalent virus infecting sweet potatoes in Kaduna State of Nigeria (Mohammed, 2018) and was attributed to higher population of whitefly (*Bemisia tabaci*) as observed in farmers' fields that transmit the virus. Most of the farmers in the study areas sourced their planting materials from their previous harvest. This practice might be a factor for the higher SPCFV incidence recorded in Bakori LGA of Katsina State because the planting materials from the previous harvest might be infected. SPV2 was reported in some parts of Nigeria (Alegbejo, 2015; Mohammed, 2018). SPFMV and SPCSV were reported in Ibadan, Oyo State, Nigeria (Schaefers and Terry, 1976; Winter *et al*., 1992). Kwak *et al*. (2014), reported

SPCFV for the first time in Korea. (17), reported SPCFV and SPMMV in KwaZulu-Natal province, South Africa. Mohammed (2018) reported SPFMV, SPCSV, and SPV2 in Kaduna State, Nigeria. The detection of SPCFV, SPMMV, and SPV2 in Kebbi and Katsina States of Nigeria during both seasons might be because of continuous circulation of infected vines for planting among farmers.

This study shows the presence of whitefly (*Bemisia tabaci* Genn.) that vectored SPMMV during both seasons and aphid (*Myzus persicae*) that vectored SPV2 only in the dry season. The common practice of intercropping sweet potatoes with maize, sorghum, and millet crops during the rainy season as widely observed in both States might have influenced the low whitefly population and absence of aphid observed during the rainy season in the study areas. There was higher rainfall in the study areas during the survey time (August 2020) which might also be a factor responsible for the absence of aphid and low mean whitefly population in the study areas. (Ndunguru *et al*., 2009) also reported low adult whiteflies per leaf and absence of aphids during the rainy season in farmers' sweet potato fields in Tanzania. Byamukama *et al*. (2004) trapped fewer whiteflies and aphids infesting sweet potatoes during long rain in Uganda.

Findings from this study however, showed no significant relationship between aphid population and SPV2 incidence; between whitefly population and SPMMV incidence in both States and seasons. Further studies are needed for the detection of virus(es) responsible for the symptoms observed in farmers' fields which tested negative to the three viruses investigated in this study.

CONCLUSION

This research reported *Sweet potato chlorotic fleck virus* (SPCFV), *Sweet potato mild mottle virus* (SPMMV), and *Sweet potato virus 2* (SPV2) infecting sweet potatoes in Kebbi and Katsina States with variation in their distribution. It also reported whitefly (*Bemisia tabaci*) that vectored SPMMV and aphid (*Myzus persicae*) that vectored SPV2 in the study areas. The continuous use of vines from previous harvest which might be infected by these viruses, irregular rouging, and weeding observed in some farms visited during the survey in the study areas might be one of the factors responsible for the higher incidence of the viruses recorded in Kebbi State. The incidence of these viruses even at lower percentage is significant as population buildup could lead to a disease outbreak. Awareness programs need to be organized for farmers on yield loss potential of these viruses on sweet potatoes plants and symptoms identification.

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