OCCURRENCE, DISTRIBUTION AND ALTERNATIVE HOSTS OF VIRUSES INFECTING SWEET POTATOES (*Ipomoea batatas* L.) IN KADUNA STATE, NIGERIA

By

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Declaration

I declare that the work in this dissertation entitled" Occurrence, distribution and alternative hosts of viruses infecting Sweet potatoes in Kaduna State, Nigeria" was carried out by me in the Department of Crop Protection. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma in this or any other institution.

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Certification

This Dissertation entitled "OCCURRENCE, DISTRIBUTION AND ALTERNATIVE HOSTS OF VIRUSES INFECTING SWEET POTATOES IN KADUNA STATE, NIGERIA" by Rahmatu Nnagiagba, MOHAMMED meets the regulations governing the award of the degree of Master of Science in Crop Protection of the Ahmadu Bello University.

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Dedication

I dedicate this work to my late sister HAJIA FATIMAH MOHAMMED for her love and words of wisdom.

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Abstract

Viruses have been reported to be one of the factors hindering sweet potato production. *Sweet potato feathery mottle virus* and *Sweet potato chlorotic stunt virus* were first reported in Ibadan Nigeria. These were reported and found to be responsible for the severe *Sweet potato virus disease* (SPVD). These viruses had been reported to cause yield reductions of 78 % in field trials in Nigeria. Information on these viruses in Northern Nigeria and Kaduna State in particular where sweet potato is largely grown is not available. Therefore, field surveys were conducted to determine the occurrence, distribution and alternative hosts of viruses infecting sweet potatoes in six Local Government Areas of Kaduna State. In July 2016, survey was conducted on sweet potatoes and weed plants in the wet season. A second survey for weed hosts was done in January 2017 dry season. The Local Government Areas visited are Giwa (Sabon-Gida, Hayin-Safiu and Halkama), Igabi (Lambakau, Fanguruzan and Tumbau), Kachia (Angwan-Ayuba, Sakwai and Gwame), Kudan (Jaja, Angwan-Sako, Dumiga), Soba (Farin-Kasa, Tabasariki and Sambirni) and Zangon-Kataf (Lenak, Zonkwa and Samaru- Kataf). Eighteen (18) farms were surveyed. In each LGA, three farms were visited. In each farm, seven (7) sweet potato leaf samples were collected making a total of 126 samples for all the six Local Government. In both wet and dry season surveys, 3 weed plants was collected per farm making a total of 108 weed samples for all the six Local Government Areas. Sweet potato leaves and weeds with and without symptoms were analysed for detection of Sweet potato viruses. Triple Antibody Sandwich Enzyme-Linked Immunosorbent Assay (TAS- ELISA) was used for the detection of SPCSV while Double Antibody Sandwich Enzyme-Linked Immunosorbent Assay (DAS- ELISA) was used for the detection of SPFMV and SPV2. The three viruses were identified in single and mixed infection for the first time on sweet potatoes and weeds in Kaduna State Nigeria. Based on the result obtained

in the laboratory analysis, Giwa LGA farms had a very high incidence of SPCSV (100%) while other viruses and their mixed infections were not detected. In Kudan LGA farms, percentage incidences of SPCSV was 70%, SPV2 (11%), SPFMV (4%), SPFMV + SPCSV (4%), SPCSV + SPV2 (11%) were identified, other mixed infections were zero. For Soba LGA farms, incidence of SPCSV was 72%, SPV2 (10%), SPCSV+SPV2 (29%) were detected. Igabi LGA farms recorded percentage incidence of SPCSV was 39%, SPFMV (22%), SPV2 (10%), SPFMV + SPCSV (11%), SPCSV + SPV2 (10%), SPFMV + SPV2 (4%), and triple infections of SPFMV +SPCSV + SPV2 (4%). Kachia LGA farms had incidences of SPFMV 62%, SPCSV (16%), SPV2 (9%), SPFMV + SPCSV (9%), SPFMV + SPV2 (4%). While Zangon-Kataf LGA farms recorded SPFMV incidence was (39%), SPCSV (31%), SPV2 (22%), SPFMV + SPCSV (8%) in percentage. The alternative host detected for SPFMV was the weed Morning glory, *Ipomoea hederaceae* L. of the family Convolvulaceae.

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List of Virus Abbreviations

Abbreviation	word in full
1. SPFMV	*Sweet potato feathery mottle virus*
2. SPCSV	*Sweet potato chlorotic stunt virus*
3. SPV2	*Sweet potato virus* 2
4. SPMMV	*Sweet potato mild mottle virus*
5. CMV	*Cucumber mosaic virus*

CHAPTER ONE

INTRODUCTION

1.1 Background to Study

Sweet potato (*Ipomoea batatas* (L.) Lam) is a dicotyledonous plant in the family *Convolvulaceae.* In this family, there are more than 1000 species with approximately 50 genera. Out of 400 species which make up the genus *Ipomoea*, only *I. batatas* is edible (Huaman, 1992). Common names are batata, sweet potatoes (tuberous morning glory in English), yam in the US, patates douce in French, Comote in Spanish etc. In Nigeria it is called "Dankali" in Hausa, "Duku" in Nupe, "Anama" in Yoruba, and "Nduku" in Igbo. It is cultivated mainly in the tropical and sub-tropical regions for its fibrous, edible, sweet and starchy tubers. It was first cultivated 5000 years ago in the tropical areas of Americas where it originated from and spread throughout the regions. It is wide spread in Europe, Asia, China, and Peru (Austin, 1988).

Huaman (1992) described sweet potato as an herbaceous perennial plant and as an annual plant by vegetative propagation using either storage roots or stem cutting. It is a prostrate plant with a vine system which extends horizontally with different types of growth habits which are either erect, semi-erect, spreading or very spreading. The root systems are fibrous for absorption of nutrient and water and storage root system for the synthesis of photosynthetic products. Depending on the variety, the tuber skin can be red, purple, brown, creamy or white. The stem is cylindrical in length and the leaves are simple and spiral in arrangements with colour varying from green-yellow, green and at times with purple pigments which are very high in anthocyanin (mostly the cyanidin type), some varieties do or do not produce flowers. Sweet potato is rich in carbohydrates, protein, minerals

(potassium, manganese, zinc, iron, phosphorus and calcium) and vitamins (A, mostly orange pulp, C, K, B1, B2, B3, and B6). The vines contain 57-78 % water of fresh weight. The carbohydrate content of the tubers include starch (13-33 %), sucrose (2.6 - 6.0 %), reducing sugar (0.3 – 0.8%), minerals (0.8 -2.2%) protein (0.8 - 2.2 %) and cellulose (0.9-2%). Carotene content of tubers varies between 0 - 24 mg, ascorbic acid (vitamin C) ranges from 23 - 43 mg/100 g in fresh tuber (USDN, 2014). It can be boiled, fried, or roasted for consumption. The fodder is used for animal feed, and the flour can be used for baking in place of cassava or wheat flour. Industrially it is processed for production of starch, syrup, alcoholic beverages, protein enriched pulp, carotene feed, yeast silage and flour. Being a rich source of vitamin A, it is also used in the pharmaceutical industries (Zhang and Li, 2004; Kapinga *et al*., 2007). In Nigeria, sweet potato is one of the four major root and tuber crops after cassava, cocoyam and yam with production output of 3,464,135.00 metric tons (FAO, 2016).

Sweet potato is reported to be infected by viruses which can either be in single or mixed infection with the dual infections of *Sweet potato feathery mottle virus* (SPFMV) and *Sweet potato chlorotic stunt virus* (SPCSV) the most commonly detected. These viruses are mostly spread by the use of vine cutting from infected mother plant, through plant to plant by sap sucking aphids and white fly (Stathers *et al*., 2005). Some of the viruses reported to infect sweet potatoes include *Sweet potato feathery mottle virus* (SPFMV) infect the crop worldwide (Moyer and Salazar, 1989), *Sweet potato chlorotic stunt virus* (SPCSV) (Karyeija *et al.,* 2000; Gibson and Aritua, 2002), *Sweet potato virus G* (SPVG) (Colinet *et al*., 1994), *Sweet potato mild mottle ipomovirus* (SPMMV) (Hollings *et al*., 1976), *Sweet potato chlorotic fleck virus* (SPCFV), *Sweet potato latent virus* (SPLV), *Sweet potato*

caulimo-like virus (SPCaLV), *Cucumber mosaic virus* (CMV) (Cohen *et al*., 1988), *Sweet potato leaf curl virus* (SPLCV), *Sweet potato ring spot virus (*SPRSV), *Sweet potato virus Y* (SPVY) (Ateka *et al*., 2004) and a host of many others. The dual infections of SPFMV and SPCSV was first reported in Ibadan Nigeria by (Schaefer and Terry, 1976) and later by winter *et al*., 1992). It is reported to be responsible for the severe *Sweet potato virus disease* (SPVD). Gibson *et al.* (1988) reported SPVD to be the major viral disease of sweet potato in East Africa. Also co-infections of SPFMV, SPCSV and SPMMV were detected in Argentina and shown to cause *Sweet potato chlorotic dwarf disease* (SPCDD) (Di feo *et al*., 2000). *Sweet potato mild mottle virus* (SPMMV) and *Sweet potato chlorotic stunt virus* (SPCSV) are transmitted by whitefly predominantly *Bemisia tabaci*, while *Sweet potato feathery mottle virus* (SPFMV) and the related *Sweet potato virus* 2 (SPV2) are transmitted by Aphids (Schaefer and Terry, 1976; Clark and Moyer, 1988). Symptoms of SPVD include stunting of plants with small leaves often distorted, narrow strap-like and crinkled, with a chlorotic, mosaic and or vein clearing symptoms but it varies with cultivar. Affected sweet potato plants generally appear pale. Yield losses of up to 90% have been reported in plants infected with SPVD (Gutierrez *et al*., 2003; Hahn, 1976; Ngeve, 1990).

1.2 Justification of the Study

Sweet potato is one of the world's most important food crops. Orange-fleshed sweet potatoes are particularly nutritious, ranking highest in nutrient contents of all vegetables for vitamins A and C, iron, copper, calcium, and fiber (CIP, 2014). It can be boiled or steamed, baked or fried and used for human food and animal feed as the root contains 16% of starch and 4% of sugar. It is used for the production of industrial starch, syrup and alcohol (USDN, 2014). Yield obtained per hectare in Kaduna State is approximately 12,000,000 Mt/Annum

(NAERLS, 2014). Abdulkareem *et al*. (2015) reported (5.5-7.4 t/ha) of sweet potatoes produced in Zaria Local Government Area of Kaduna State. Average yield of 3,400,000 Mt /Annum is recorded in Nigeria and 77,375,000 Mt /Annum in China (FAO STAT, 2016). Viruses, however have been implicated to be one of the limiting factors in sweet potato production. SPFMV and SPCSV were first reported in Ibadan Nigeria by (Schaefer and Terry, 1976) and later by winter *et al*. (1992). These viruses had been reported to cause yield reductions of 78 % in field trials in Nigeria (Hahn, 1979). In Uganda, yield loss of 14-52% was recorded for SPCSV in single infection while in mixed infection with SPFMV, 60- 95% yield loss was recorded (Gibson *et al*., 1998). Research on viruses infecting sweet potatoes and its reservoir hosts have not been documented in Kaduna State being one of the major producing States in Nigeria. There is therefore the need to have information on the incidence and distribution of viruses infecting sweet potatoes and to determine the alternative hosts which may serve as reservoir hosts of these viruses. This study was therefore conceived with the following objectives.

1.3 Objectives of the Study

The objectives of this research are to determine:

i. the incidence and distribution of viruses infecting sweet potatoes in Kaduna State.

ii. the alternative hosts of the viruses.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 The Sweet Potato Plant

2.1.1 Origin and distribution of sweet potato

Austin (1988), and Yen (1982) reported that sweet potato (*Ipomoea batatas* L. Lam) was first domesticated 5000 years ago in tropical America based on the analyses of key morphological characters of sweet potato and the wild *Ipomoea* species . He also postulated that sweet potato originated in the region between the Yucatan peninsula of Mexico and the Orinoco River in Venezuela (Austin, 1988). According to Huang and Sun (2000), Zhang *et al.*, (2000), the primary centre of diversity and most likely the centre of origin of sweet potato were hypothesized to be the Central America. Based on this, molecular marker technique was used and Central America was recorded to have the highest centre of diversity which supports the hypothesis. The Columbus in 1492 took it to the Europe and Portuguese explorers of the Sixteenth Century took it to Africa, India, Southeast-Asia and East Indies. The Spanish ship was reported to transport it to Philippines from Mexico in the 16th century. In Nigeria, production spread all over the country. In Nigeria, the major producing areas include Niger, Kwara, Plateau, Benue, Abuja, Akwa-ibom, Imo, Ebonyi, Nassarawa, Benue and Kaduna where most of the crop is produced (Tewe *et al*., 2003).

2.1.2 Taxonomy and morphology of sweet potatoes

Ipomoea batatas belongs to the family Convolulaceae which in English it means morning glory. Linnaeus (1753) described the cultivated sweetpotato as *Convolvulus batatas*. In 1791, the botanist Lamarck described it as *Ipomoea batatas*. It is a hexaploid plant with

2n=6x=90 chromosomes. Some plants that are morphologically similar to *I. batatas* with 2n=4x=60 chromosomes have been described and named; they are considered synonyms of this species (Austin, 1978). *Ipomoea batatas* is a self-incompatible species. In the family *convolvulaceae* there are approximately 50 genera and more than 1000 species and *Ipomoea* is a large genus composed of approximately 400 species.

2.1.3 Botanical description of sweet potatoes

Huaman (1992) described the sweet potato plant as an herbaceous perennial plant. And as an annual plant by vegetative propagation using either the storage roots or stems cuttings. The growth habit is prostrate with a vine system which extends horizontally on the ground. It can also be erect, semi-erect, spreading and very spreading. The roots are classified as fibrous and storage (lateral roots). The fibrous roots absorb water, nutrient and anchor the plant while the storage root stores photosynthetic products. The storage root is the commercial part of sweet potato and consists of the proximal end (attach to the stem) which produces the adventitious buds and sprout and the distal end. The stem of *Ipomoea batatas* is trailing or twining which can either be prostrate or ascending, often twining, glabrous or pubescent and light green to purple in color. The long, thin stem that creep on the surface produce extensive, fibrous roots where nodes make contact with the soil. Tuber flesh color may be white, yellow, orange, reddish or purple. Stem length ranges from 1 to 5 m, depending on cultivar or variety.

Leaves are simple and arranged spirally with petioles measuring between 5 and 30 cm long. The leaf lamina is mostly ovate and can be entire to deeply digitately lobed, with their base usually cordate. The leaf tips can be acute or obtuse and the leaves can be glabrous, or with variable pubescence with color varying from light green to deep purple, sometimes with

purple stain at their base, or with green or purple veins beneath. Flower colour can either be white, pink or deep purple depending on the variety which are solitary or in clusters of buds; flower has five sepals, five petals, five stamens, a compound pistil, and fused corolla. Five stamens are attached at the base of the corolla and are of variable length. The ovary is two-celled; contains up to four seeds, but usually only one or two are fully developed (Huaman, 1996).

2.1.4 Production of sweet potato

Nigeria is the leading sweet potato producing countries in Africa followed by Tanzania (FAOSTAT, 2016). Osun, Benue, Kaduna, Kwara, Plateau and Abuja are reported to be the major States of sweet potato cultivation in Nigeria with yield output of 3,464,135.00 tons Nationwide after China according to Table1. In 1994 the United Nation Food and Agriculture Organization estimated the area of sweet potato crop cultivation in Nigeria at 69,000 hectares which have increased to over 500,000 hectares in 2003 (FAOSTAT, 2004). In Kaduna State of Nigeria, 107, 33 hectare of land was used for sweet potato cultivation with production output of 1,035.4 Metric tonnes (NAERLS, 2014). It is also grown on about 8.2 million hectares, yielding about 102,697,894.00 tons worldwide (FAO, 2016).

Table 2.1. Top ten Sweet potato producing countries in the world

Rank	Country	Production (MT)
1	China	77,375,000
2	Nigeria	3,400,000
3	Uganda	2,645,700
4	Indonesia	2,483,467
5	Tanzania	3,018,175
6	Vietnam	1,422,501
7	Ethiopia	1,185,050
8	United States of America	1,201,203
9	India	1,072,800
10	Rwanda	1,005,305

Source: FAO (2016).

2.1.5 Cultivation of sweet potato

Sweet potato plant grows best at an average temperature of 24 °C, abundant sunshine and warm nights with altitude of 1700 m above sea level, the taste and dry matter content might be poor if grown on altitude of up to 2500 m (Stathers *et al*., 2005). The plant requires annual rainfalls of 750 - 1,000 mm, with a minimum of 500 mm in the growing season on well-drained, light- and medium-textured fertile sandy loamy soils with a pH range of 5.6-6.6 on moulds, ridges or bed for good drainage and aeration. Roots mature in two to nine months. It is mostly propagated by stem, root cuttings or by adventitious roots called "slips" that grow out from the tuberous roots during storage. The vine cuttings sprout should have at least 3 nodes 20-30 cm with 25-30 cm spacing between plants and 60-100 cm between ridges (Stathers *et al*., 2005). It can be grown in poor soils with little fertilizer, very sensitive to aluminum toxicity. Ash is applied to substitute for potassium as it requires potassium to

produce high quality roots, with less nitrogen. Sweet potatoes can also be interred- cropped with pigeon pea maize and millet (Stathers *et al*., 2005).

2.1.6 Nutritional composition of sweet potato

Sweet potato root is an excellent source of vitamin A (for proper vision and immunity), most especially the orange flesh color, Vitamin C (essential for growth, tissue repair, wound healing and cartilage bone and teeth maintenance), vitamin B6, Riboflavin, Copper, Panothenic acid and folic acid. It also contains considerable amount of Iron (boost energy), starch, and sodium among others (USDN, 2014). The iron present makes it a good recommendation for women in gestation (Wilmer *et al*., 2014). According to a research carried out on the treatment of cervical cancer, it was reported that the fresh leaves of purple sweet potato would be helpful in combating the disease in its early stage due to its high concentration of anthocyanin (cyanidin type) (USDN, 2014). (Burri, 2011) stated that in Nigeria, the orange-fleshed sweet potatoes contains beta-carotene that helps fight vitamin A deficiency, and good for pregnant women and children. Beta-carotene, the Vitamin A that is found abundantly in sweet potatoes has been shown to reduce asthma symptoms. The storage root contains dietary fiber (pectin, cellulose, hemicellulos and lignin), protein, vitamin (Beta-carotene, vitamin B1, B2, Vitamin C, and E), Minerals (K, Fe, Ca) Energy and Carbohydrate. The root and skin contain high level of polyphenols such as Anthocyanins and phenolic acids e.g. caffeic acid. Chlorogenic dicaffeoyl quinic and tricaffeoyll quinic acid are derivatives of caffeoylquinic acid that protect the root from fungal diseases and have potential cancer chemo- protective effect (Kansas *et al*., 2009).

2.1.7 Uses of sweet potatoes

Sweet potato is mainly consumed as carbohydrate food by man and animals. Many parts of sweet potato plant, (leaves, roots and vines) are edible. It can be used industrially for production of starch (24-26 %) which is processed into flour for baking, as stabilizer in ice cream industries, canned (mostly the yellow fleshed variety), as animal feed (leaves), beverages, beer and starch (bread, syrup, isomerizes glucose). In Nigeria, peeled and dried sweet potato roots are use as sweeteners in beverages (Kunu zaki) Odebode (2004). Purple sweet potato colour is used as food coloring. Some cultivars are used in gardens as ornamental plants for their attractive foliage. The vines are used for home aquariums .The numerous acylated anthocyanins are the major colour constituents in the storage roots and used for treatment of diabetics (USDN, 2014). In South America, the juice of red sweet potato is combined with lime juice to make dyes for cloth (Duke and Wain, 1981).

2.2 Production Constraints of Sweet Potato

Fawole (2007) outlined the constraints of sweet potato production which include inadequate government aid, cost of labor, poor access to credit, poor storage facilities, lack of new technologies, poor market outlets, high incidence of insect pests and diseases, lack of improved practices, poor storage facilities, lack of credit facilities, lack of extension training, and poor transportation. Insect pests such as sweet potato weevils, sweet potato aphids (*Aphis gossypii* Glove rand *A. craccivora* Koch) and mites cause damage on sweet potato**.** Both adult and larva of these pests attack tubers, foliage and stems causing a significant yield loss in production. Diseases, mostly sweet potato virus disease (SPVD) caused by synergistic interaction of S*weet potato chlorotic stunt virus disease* (SPCSV) and *Sweet potato feathery mottle virus* (SPFMV) are the most severe. Gutierrez *et al*.

(2003) stated that SPCSV alone can lead to mild to moderate symptoms with yield loss of up to 43 % depending on cultivar, infecting virus, stage of infection and whether the crop is infected with a single or multiple viruses. Viral diseases may cause up to 100 % yield loss (Gibson *et al*., 1997).

2.3 Diseases and Insect Pests of Sweet Potato

Sweet potatoes are attacked by a wide range of insect pests and diseases. They affect vascular tissue as well as storage and fibrous roots, causing vine wilting and rots. According to Ames *et al*., (1997), bacterial stem and root rot (*Erwinia chrysanthemi*), bacterial wilt (*Pseudomonas ralstonia*), soil rot (*Streptomyces ipomoea*), leaf and stem scab (*Elsinoe batatas, Sphaceloma batatas*), are some of the devastating bacteria diseases attacking sweet potato worldwide. The fungus which causes *Alternaria* disease with varying levels of damage has also been reported to be one of the major challenges to output of sweet potato (Clark, 1987). The main harmful insect pests are the sweet potato weevils of the genus *Cylas* (*C. puncticollis* and *C. brunneus*) (Purseglove, 1968). *Cylas formicarius* is mainly distributed over East Africa. It attacks the leaves, stems and roots (Skoglund and Smit, 1994). Other minor insect pests are sweet potato butterfly (*Acraea acereta*) caterpillars and mites (Skoglund and Smit, 1994). Most common viral diseases include *Sweet potato chlorotic stunt virus* (Whitefly-transmitted crinivirus), *Sweet potato feathery mottle virus* (SPFMV) (Aphid-transmitted potyvirus), *Sweet potato sunken vein virus* (SPSVV) (Whitefly-transmitted Closterovirus), *Sweet potato virus disease* (SPVD), *Sweet potato mild mottle virus* (SPMMV) (Whitefly-transmitted potyvirus) and a host of many others (Fuglie, 2007).

2.4 Sweet Potato Infecting Viruses

A suspected virus disease of sweet potato was reported in 1944 in southern Carolina but 20 viruses have been recently reported to infect sweet potato (Fuglie, 2007). These include the *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), *Sweet potato caulimo-like virus* (SPCaLV), *Cucumber mosaic virus* (CMV) and *Sweet potato leaf curl virus* (SPLCV). There are also other viruses reported to infect sweet potato: *Sweet potato leaf speckling luteovirus* (SPLSV), *Sweet potato latent potyvirus* (SPLV), *Sweet potato ring spot nepovirus* (SPRSV), *Sweet potato caulimovirus* (SPCaLV), *Sweet potato yellow dwarf ipomovirus* (SPYDV), *Sweet potato vein mosaic potyvirus* (SPVMV), *Sweet potato leaf curl badnavirus* (SPLCV), *Sweet potato leaf curl geminivirus*-US (SPLCV-US), *Ipomoea crinkle leaf curl geminivirus* (SPCLCV), and *Sweet potato phytoreovirus* SPP (Chavi *et al*., 1997; Gibson *et al*., 1997).

2.4.1 Sweet potato feathery mottle virus (SPFMV)

It belongs to the family: potyviridae genus: potyvirus. It was previously named as *Sweet potato chlorotic leaf spot virus, Sweet potatoes internal cork virus, Sweet potatoes russet crack virus, Sweet potatoes vein mosaic virus, Sweet potatoes virus A, Sweet potatoes vein clearing virus and Sweet potato ring spot virus* (Karyeija *et al.*, 1998). It is transmitted non-persistently by Aphids (*Myzus persicae* Sulzer and *Aphis gossypii* Glover). It has a flexuous and elongate virion with a monopartite single- stranded, linear RNA molecule. In the cytoplasm, lengths of 830-850 nm particles are present in all parts of the host plant with wheel pin inclusion bodies present in infected cells which is similar to that of the potyviridae

(Cohen *et al.*, 1997). Thermal inactivation point is 60-90 ^{O}C, longevity *in-vitro* 0.3 to 0.5 days or 7-12 hrs, dilution end point 10^{-3}-$10^{-4.9}$ (Alegbejo, 2015).

The virus is seed-borne and disseminated in tubers and vines (Cadena and Campbell, 1981). It is rare to recognize this virus visually on the farmers' field. There are different isolates of these virus and some cause loss in yield by affecting the quality of the storage root. They can cause internal cork rots and russet crack of the storage root. The symptom of this virus is either on the leaf or the tuber. On the leaf the symptoms might be mild or absent. The leaf appears to be faint with irregular chlorotic spots and also with purplish stains at times. These chlorotic patterns are usually feathery either along the midribs or around the leaf. These chlorotic spots might be bordered with some purple pigment as well but not often (Ames *et al*., 1997). For the symptoms to be very visible, the cultivar of sweet potatoes must be susceptible, or if the plant is under stressed for water and nutrient supply, the growth stage of the plant and also the strain of the virus either virulent or a virulent. There are 3 types of strain of this virus which affect the storage root and the expression of symptoms has to do with the strain of the virus and the variety of sweet potatoes. The strain can be the common strain which usually does not show visible symptoms, the russet crack strain and internal cork stain (external and internal dark necrotic lesions) (Karyeija *et al*., 1998, 2001). This virus is found to occur worldwide and reported to cause yield reduction of over 50 %, if the cultivar is susceptible (Stathers *et al.,* 2005). The main host is the sweet potato plant and some wild *Ipomoea* species. Other weed hosts which are grouped into 3 families include Chenopodiaceae: *Chenopodium Murale* L, *C. amaranticolor* Coste, *C. quinoa* L. and several strains of *Spinacia Oleracea* L. Convolvulaceae family host include; *Calonyction aculeatum* L., *Ipomoea hederaceae* L., *Ipomoea incarnate* L, *I. lacunose L., I. tricolor* Car,

I. wrightii Choisy etc. Solanaceae: *Datura metel* Ber., *Nicotiana benthamiana* Domin., *N. occidentalis* Whaler., *N. clevelandii* Gray (Thompson and Myndardt, 1986).

2.4.2 Sweet potato mild mottle virus (SPMMV)

In East Africa, sweet potato plants were showing leaf mottling, veinal chlorosis, dwarfing and poor growth (Sheffield, 1957). After a test, (Brunt *et al.,* 1996) found that this was caused by SPMMV. It is in the family Potyviridae, genus Ipomovirus. It is an RNA containing virus with filamentous particle 950 nm long and induces a cytoplasmic pinwheel inclusion which is similar to that of the potyviridae. It is transmitted manually by grafting and by whitefly (*Bemisia tabaci* Genn*)* in a persistent manner and not by contact between plants and seeds (Hollings *et al*., 1976; Moyer and Salazar, 1989; Brunt *et al*., 1996). This virus is reported to cause reduction in vine quality by producing mild mottling of leaf, distinct venial chlorosis on *Ipomoea setose* and stunting (Brunt *et al*., 1996; Ames *et al*., 1997). It is reported to be spread in East Africa. Its natural host is Sweet potato plant. Other hosts include *Chenopodium quinoa* (Wild.), *C. murale* L., *Beta vulgaris* L., *Datura stramonium* L., *Nicotiana tabacum* L., *Nicotiana benthamiana (*Domin), *Nicotiana. glutinosa* L. (Aritua *et al*., 2002; Ateka *et al*., 2002) reported that this virus was found in Uganda and Kenya at low incidences in single and multiple infections with other sweet potato viruses.

2.4.3 Sweet potato chlorotic stunt virus (SPCSV)

Previously, this virus was known as *Sweet potato sunken vein virus*. It is a bipartite whitefly-transmitted virus in the genus *Crinivirus* (Cohen *et al*., 1992). It is mostly found in the phloem of sweet potato plant and transmitted in a semi-persistent manner by *B. tabaci* and *Trialeuroda abutilonea Haldeman* (Sheffield, 1957). Host plants of SPCSV include

various *Ipomoea* species, *Nicotiana spp, Benthamiana spp*, *N. clevelandii*, and *Amaranthus palmeri* (Cohen *et al*., 1992). Under favorable disease conditions this virus causes mild symptoms in sweet potato cultivars which are slight stunting; purpling of the lower leaves and mild chlorotic mottle of the middle leaves (Winter *et al*., 1992; Gibson *et al*., 1998). However, it causes a severe disease in sweet potato when present in mixed infections with some strains of SPFMV (Cohen *et al*., 1992; Gibson *et al*., 1998; Karyeija *et al*., 2000). SPCSV is known to form synergetic complex with other viruses such as SPFMV and SPMMV (Schaefers and Terry, 1976 Gibson *et al*., 1998; Di feo *et al*., 2000; Gibson and Aritua; 2002) and distributed in Nigeria, Zambia and Tanzania (Gibson *et al*., 1998; Katisha and Gibson, 1999).

2.4.4 Sweet potato virus disease (SPVD)

This is used to describe plants that are infected by dual infections of *Sweet potato chlorotic stunt virus* and *Sweet potato feathery mottle virus* (Gibson and Aritua, 2002). It was first reported in Eastern Belgian Congo in 1939 and reported in Nigeria Schaefers and Terry (1976). Combined infections of SPFMV and SPCSV result in the development of SPVD characterized by chlorotic mottling in which the whole plant look pale, leaves are small and narrow (strap like or fanlike) often with distorted edges, vein clearing, crinkled leaves, and severe stunting (Gibson and Aritua, 2002).These viruses synergistically break down the resistance of sweet potato against *Sweet potato feathery mottle virus* and helps SPFMV to multiply. The mechanism of synergisms has not been detected. SPCSV is also reported to cause synergisms of sweet potato with other viruses like *Cucumber mosaic virus, Sweet potato mild speckling virus* etc. SPVD causes yield loss up to 90% mostly in the orange

colour cultivars. The incidence is reported to be higher in fields planted as mono-crop than in the other cropping patterns (Ndungum and Alonge, 2000).

2.4.5 Cucumber mosaic cucumovirus (CMV)

First observed in 1986 infecting sweet potato in Israel and United States of America (Ames *et al.,* 1997). The CMV infected plants were also infected with SPFMV (Cohen *et al*., 1988; Moyer *et al.,* 1989). CMV requires a helper virus to replicate in sweet potato. It is transmitted to sweet potato mechanically and by aphids if the acceptor plant carries the whitefly (*Bemisia tabaci*) transmitted virus which helps in replication.

2.4.6 Sweet potato virus 2 (SPV2)

Is a potyvirus in the family potyviridae. Members of these families are characterized molecularly as positive sense single stranded RNA virus with a genome of approximately 10kb with a 5' untranslated region (UTR), a large open reading frame (ORF) and a 3' UTR. The ORF consists of 10 functional proteins P1 (Protein1), HC pro (helper component proteinase), P3 (protein 3) 6K1 (Protein 1), CI (cylindrical inclusion protein), 6K2 (6k protein 2) Vpg (viral protein genome linked), NIa- pro (nuclear inclusion protein a-proteinase), NiPb (nuclear inclusion protein b) and CP (coat protein) (Alegbejo, 2015). The virus is transmitted by aphids (*Myzus persicae* Sulz) to several species of the genera *Chenopodium*, *Datura*, *Nicotiana* and *Ipomoea* (Gibson and Aritua, 2002).

Other viruses reported to infect sweet potato includes *Sweet potato leaf speckling luteovirus* (SPLSV), *Sweet potato latent potyvirus* (SPLV), *Sweet potato ring spot nepovius* (SPRSV), *Sweet potato caulimovirus* (SPCaLV), *Sweet potato yellow dwarf ipomovirus* (SPYDV), *Sweet potato vein mosaic potyvirus* (SPVMV), *Sweet potato leaf curl badnavius* (SPLCV),

Sweet potato leaf curl germinivirus –US (SPLCV-US), Ipomoea crinkle leaf curl germinivirus (SPCLCV), and *Sweet potato phytoreovirus* also reported. Of all these viruses only SPCFV, SPRSV, SPLV and SPCaLV have been reported in Africa (Atkey and Brunt, 1987; Brunt *et al*., 1996; Carey *et al*., 1997; Gibson *et al*., 1997). Sweet potato chlorotic dwarf disease (SPCDD) a synergistic combination of SPFMV, SPMSV and SPCSV is another virus complex reported in Argentina (Di Feo *et al*., 2000; Gibson and Aritua, 2002) its symptoms on plants infected is characterized by reduced leaf area, stunting and severe mosaic of leaves, blisters and distortion.

2.5 Management of Sweet Potato Viruses

Sweet potato viruses can be managed through the selection of SPVD resistant cultivars, use of disease free planting material and removal of infected plants (Karyeija *et al.,* 1998). According to Gibson and Aritua (2002) sweet potato viruses can be controlled using virus free planting material and controlling weeds, which may serve as alternative hosts of insects and viruses, especially wild *Ipomoea* species in and around fields. Isolating new crops a distance from the old mature crops will reduce virus incidence and result in high yielding crops. Intercropping sweet potato with maize prevent SPVD incidence by delaying vectors onset and build up. If volunteer sweet potato plants, which may have survived from previous crops, are removed and resistant varieties planted, viral diseases can be minimized (Thompson and Mynhardt, 1986; Karyeija *et al.,* 1998). Stathers *et al*., (2005) also recommended that immediately disease symptoms are observed, the plants should be uprooted. Crop rotation should be encouraged because roots and cuttings from old surviving diseased plants in the soil will produce disease plants which act as source of inoculums to

the new crop. Planting of barrier crop, field hygiene and use of resistant varieties should be encouraged.

2.6 Alternative Hosts of Sweet Potato Viruses

Sweet potato chlorotic stunt virus (SPCSV) has a wide host range. Sheffield, (1957) detected it in sweet potatoes in Nigeria. In addition to sweet potatoes which is the main host, other hosts include the *Nicotiana spp, Benthamiana* spp Domin, *N. clevelandii* Gray, and *Amaranthus palmeri* L. (Cohen *et al*., 1992). *Sweet potato mild mottle virus* (SPMMV) natural host is sweet potato. Other hosts include *Chenopodium quinoa* (Wild.) *C. murale* L., *Beta vulgaris* L., *Datura stramonium* L., *I. setosa* Kerr, *Nicotiana tabacum* L., *Nicotiana benthamiana (*Domin), *Nicotiana glutinosa* L. The main host of *Sweet potato feathery mottle virus* is the sweet potatoes plant and some wild *Ipomoea* species. Other weed host which is grouped into 3 families includes *Chenopodiaceae* family*: Chenopodium murale* L, *C. amaranticolor* L, *C. quinoa* (Wild.) and several strains of *Spinacia oleracea* L. *Convolvulaceae* family host include; *Calonyction aculeatum DC., Ipomoea hederaceae* L, *Ipomoea incarnate* L, *I. lacunose* L, *I. tricolor* L, *I. wrightii* L. etc. *Solanaceae* family: *Datura metel L, Nicotiana benthamiana (*Domin), *N. occidentalis* L, *N. clevelandii* L. (Cohen *et al*., 1992).

Sweet potato mild mottle virus main host is the sweet potato plant. Other hosts include *Chenopodium quinoa* (Wild.), *C.* murale L., *Beta vulgaris* L., *Datura stramonium* L., *Nicotiana tabacum* L., *Nicotiana benthamiana (*Domin), *Nicotiana. glutinosa* L. (Aritua *et al*., 2002; Ateka *et al*., 2002). Host range of *Sweet potato virus* 2 include *Chenopodium quinoa* (Wild.), *C. murale* L., *C. quinoa* L., *Datura metel* L., *D. stramonium* L., *Nicotiana tabacum* L., *Nicotiana benthamiana (*Domin), *Nicotiana. glutinosa* L., *N clevelandii* L, *N.*

debneyi L, *N. hesperis* L., *N. oblique* L., *N. occidentalis* , *I. batata* L., *I. coccinea* L., *I. nil*, *I. purpurea* L., *I. setosa* Kerr. (Aritua *et al*., 2002).

2.7 Detection of Sweet Potato Viruses

Sweet potato viruses can be detected using bioassay method, vector transmission procedure, and serological method. Bioassay is done in two ways either by mechanical inoculation or grafting. Mechanical inoculation is the application of sap containing the virus on to the surface of leaves of an indicator plant. The virus enters the cells and symptoms are expressed on the plant (Sheffield, 1957). Grafting is done by grafting two leaf shoots of sweet potato onto *Ipomoea setose* ker and *Ipomoea nil* L. and symptoms are observed after 4 weeks (Anonymous, 1978). For the insect transmission method, insects are allowed acquisition access feeding and are kept on the acceptor plant for inoculation of the virus (Winter *et al*., 1992). Serological method is based on the covalent linkage of an enzyme to an antibody registering the occurrence of an antigen-antibody complex by rapid enzymatic development of colour. Triple antibody sandwich (TAS) , double antibody sandwich (DAS) or Nitro-cellulose membrane Enzyme Linked Immunosorbent Assay (NCMELISA) is used to confirm which virus is present against the Antisera. Polymerase chain reaction (PCR), reverse transcription PCR (RT-PCR), molecular hybridization, electrophoretic analysis of dsRNA, western blot, and serologically specific electron microscopy (Derrick, 1973; Clark and Adams, 1977; Abad and Moyer, 1992; Colinet *et al*., 1998) have also been used successfully for diagnosis. Any of these techniques can be combined to complement indexing.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Survey and sampling of sweet potato fields and alternative weed hosts of sweetpotato viruses

Survey for viruses infecting sweet potatoes and their weed hosts were conducted in Kaduna State of Nigeria in July 2016 wet season. In January 2017 dry season, a second survey was conducted on weed plants to detect the alternative hosts. Six Local Government Areas (LGAs) namely, Giwa (Sabon-Gida, Hayin-Safiu and Halkama), Igabi (Lambakau, Fanguruzan and Tumbau), Kachia (Angwan-Ayuba, Sakwai and Gwame), Kudan (Jaja, Angwan-Sako and Dumiga), Soba (Farin-Kasa, Tabasariki and Sambirni), and Zangon-Kataf (Lenak, Zonkwa and Samaru-Kataf) were surveyed (Figure 1). The farms were selected for disease incidence assessment based on sweet potato production history and advice from extension agents. A total of 18 farms were surveyed. Three fields were randomly chosen and visited per local government. In each farm, seven (7) leaf samples of sweet potato were collected making a total of (126) samples for all the six local government Areas. Virus incidence was recorded as the number of diseased plants in the total number of test samples in the study field. The percentage incidence was calculated using the formula below expressed in percentage (%).

$$\text{Virus incidence } (\%) = \frac{\text{Number of diseased plants}}{\text{Total number of plants examined}} \times 100$$

Broad and narrow leaf weed species, within and around the sweet potato fields with or without viral disease symptoms were randomly collected at a distance of 5 meters. The

survey was done in both wet and dry seasons. In each season (54) weed samples were collected making a total of (108) samples in both seasons. The weeds were taken to botany department of biological science ABU Zaria for identification. The identified weed samples were labeled and brought back to the Virology Laboratory, Department of Crop Protection, ABU, Zaria and stored at about -20˚C before analyses. A survey questionnaire was administered to the farmers visited and the following data were collected: Name of LGA and location of the farm, co-ordinates of the location of the farm, elevation, the farm size, cropping history, cropping pattern, crops surrounding the farm, crop protection practices employed, field hygiene, age of the crop, observed symptoms on both sweet potato plant and alternative hosts, source of vines, and the varieties of sweet potatoes grown (Appendix I).

The average disease incidence in each field and LGA was calculated. The coordinates of the surveyed locations were also taken using Garmin hand held Geographical Positioning System (GPS).

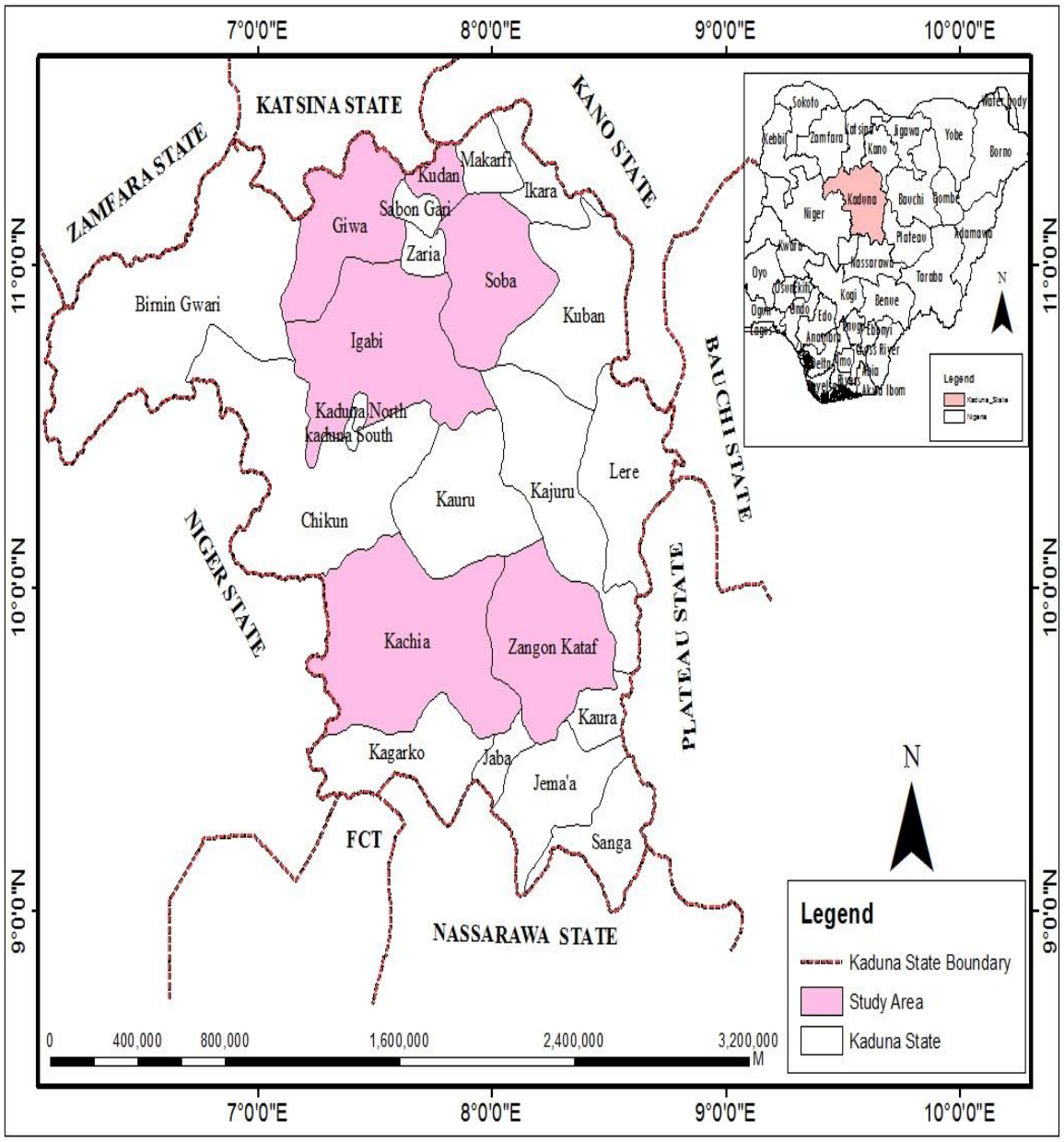


Figure 3.1: Map of Kaduna State showing the six (Giwa, Igabi, Kachia, Kudan, Soba and Zangon-Kataf) Local Government Areas visited.

3.2 Laboratory Detection of Viruses in Sweet Potato Leaf Samples and Weeds

Serological tests were carried out on the sweet potato leaf and weed samples using Double Antibody Sandwich Enzyme Linked Immunosorbent Assay (DAS-ELISA) and Triple Antibody Sandwich ELlSA (TAS-ELISA) as specified by the supplier of the detection kits Leibniz-Institute DSMZ- Deutsche Sammlung von Mikroorganismen und Zellkulturen Gmbh, Braunschweig, Germany. Each plate used contained extracts of twenty-one sweet potato leaf test sample, 9 weed test sample, positive and negative controls. Buffers used for the laboratory analysis is listed in Appendix II.

3.2.1 Procedure for double antibody sandwich enzyme-linked immunosorbent assay (DASELISA) for *Sweet potato feathery mottle virus* (SPFMV) and *Sweet potato virus 2* (SPV2) detection

DAS-ELISA was carried out as described by the supplier of the detection kits Leibniz-Institute DSMZ- Deutsche Sammlung von Mikroorganismen und Zellkulturen Gmbh, Braunschweig, Germany. The microtitre ELISA plates was coated using polyclonal antibody-IgG for *sweet potato feathery mottle virus* and *sweet potato virus 2* in coating buffer in a dilution of 1:1000. 200 µl was dispensed into each well of the microtitre plate. The plates were incubated at 37 ˚C for 2 h. At the end of the incubation period, the plates were washed three times with phosphate buffer saline (PBS-Tween 20) using a wash bottle by soaking for few minutes before emptying them. The plates were then blotted by tapping twice on a multiple layered tissue paper.

The test samples were homogenised (1:10 w/v) using a sterile mortar and pestle in sample extraction buffer (PBS-Tween 20 and 2 % PVP-Serva) and 200 µl were pippeted into each

test well. The plates were incubated overnight at 4 ˚C and thereafter washed with PBS-Tween 20 as stated above. The anti-virus conjugate (IgG-AP) was diluted in conjugate buffer at 1:500 and 200 µl was pipetted and dispensed into each well. The plates were incubated at 37 ˚C for 2 h, and thereafter washed with PBS-T as earlier described. Freshly prepared substrate aliquots (10 mg p-nitrophenyl phosphate [Sigma, Fluka] dissolved in 10 ml of substrate buffer [97 ml diethanolamine, 0.2 g NaN_3, 600 ml H_2O, pH 9.8]) were added to each well (200 µl/well). The plates were then incubated in the dark at room temperature for 60 minutes for visual observation. Finally, the plates were read using Uniequip ELISA plate reader at $A_{405\ nm}$ absorbance. Readings that were twice the values of the healthy controls were considered as positive (Kumar, 2009).

3.2.2 Triple Antibody Sandwich Enzyme-Linked Immunosorbent Assay (TAS ELISA) for *sweet potato chlorotic stunt virus* (SPCSV) detection

TAS-ELISA was done as described by the supplier of the detection kits Leibniz-Institute DSMZ- Deutsche Sammlung von Mikroorganismen und Zellkulturen Gmbh, Braunschweig, Germany. Each well of microtitre plates were coated with *Sweet potato chlorotic stunt virus* polyclonal antibodies (200 µl/well) which were diluted in coating buffer at a ratio of 1:1000. The plates were incubated for 2 h at 37 ˚C, and washed with PBS-T and blotted on a multi-layered tissue paper repeated three times. Unbound sites well plates were blocked by adding 200 µl 2 % skimmed milk which was dissolved in PBS-T, into each well. The plates were incubated for 30 minutes at 37 ˚C, drained and tap dried on a tissue paper but not washed. The test samples were homogenised (1:10 w/v) using a sterile mortar and pestle in sample extraction buffer (PBS-T and 2 % PVP-Serva) after

which (200 µl/well) was pipetted and dispensed into the test wells. The plates were then incubated overnight at 4 ˚C and were washed with PBS-T after the incubation period as stated above. Two hundred microlitres of SPCSV monoclonal antibodies dissolved in a dilution of 1:1000 of conjugate buffer were added in to each test well and incubated for 4 h at 37 ˚C. Thereafter, the plates were washed with PBS-T as previously described. Rabbit anti-mouse alkaline phosphate (RAM-AP) conjugate, diluted in 1:1000 was added into each well (200 µl). The plates were incubated for 2 h at 37 ˚C and washed with PBS-T three times as stated above. Two hundred micro litres aliquots of freshly prepared substrate (10 mg p-nitrophenyl phosphate, PNPP [Sigma, Fluka] dissolved in 10 ml of substrate buffer was added to each well. It was then incubated at room temperature for 60 minutes for visual observation. The plates were then, read using Uniequip ELISA plate reader at $A_{405\ nm}$ absorbance. Readings that were twice the values of the healthy control were considered as positive (Kumar, 2009). Simple percentage analysis was used to analyze the results that were collected.

3.3 Data Analysis

Data collected on the virus disease incidence from all the six Local Government Areas were analyzed using descriptive statistics (bar chart) on Microsoft Excel.

CHAPTER FOUR

4.0 RESULTS

4.1 Incidence of Sweet potato viruses

4.1.1 Virus incidence in Giwa, Kudan, Soba, Igabi, Kachia and Zango-Kataf

The mean virus incidence detected in the six local Government Areas of the State is shown in Figures (4.2 A- F). The incidence of SPCSV was very high (100 %). It was the only virus detected in Giwa Local Government Areaa of Kaduna State (A). In Kudan Local Government Area, SPCSV, SPV2 and SPFMV incidence were 70%, 11%, and 4%, respectively. The mixed infections detected were SPFMV + SPCSV (4%) and SPCSV + SPV2 (11%) (B). In Soba Local Government Area, SPCSV incidence was 72.0% and SPV2 10.0%. Mixed infections detected was SPCSV + SPV2 (29%) (C) were found. In Igabi Local Government Area, SPCSV incidence was (39.0%), SPFMV (22.0%) and SPV2 (10%). Double infections of SPFMV + SPCSV (11%), SPCSV + SPV2 (10%), SPFMV+SPV2 (4%) were found, while triple infection of SPFMV+SPCSV+SPV2 (4%) (D) was detected. In Kachia Local Government Area, incidence of SPFMV was 62.0%, SPCSV 16.0% and SPV2 (9%). Mixed infections of SPFMV + SPCSV (9%), SPFMV+SPV2 (4%) (E) were detected. In Zangon-Kataf Local Government Area, SPFMV had incidence of 39.0%, SPCSV 31.0% and SPV2 22%. Mixed infection of SPFMV + SPCSV (8%) (F) was detected.

The surrounding crops, sanitary condition, stage of crop growth and cropping pattern in each field are shown in Appendix III. Plate 1A is showing a healthy sweet potato leaf sample collected during the survey. The different symptoms observed in the locations were diffuse

chlorotic spots, purpling, severe leaf distortion,vein clearing, mottlings, and interveinal chlorosis (Plates 1B to D).

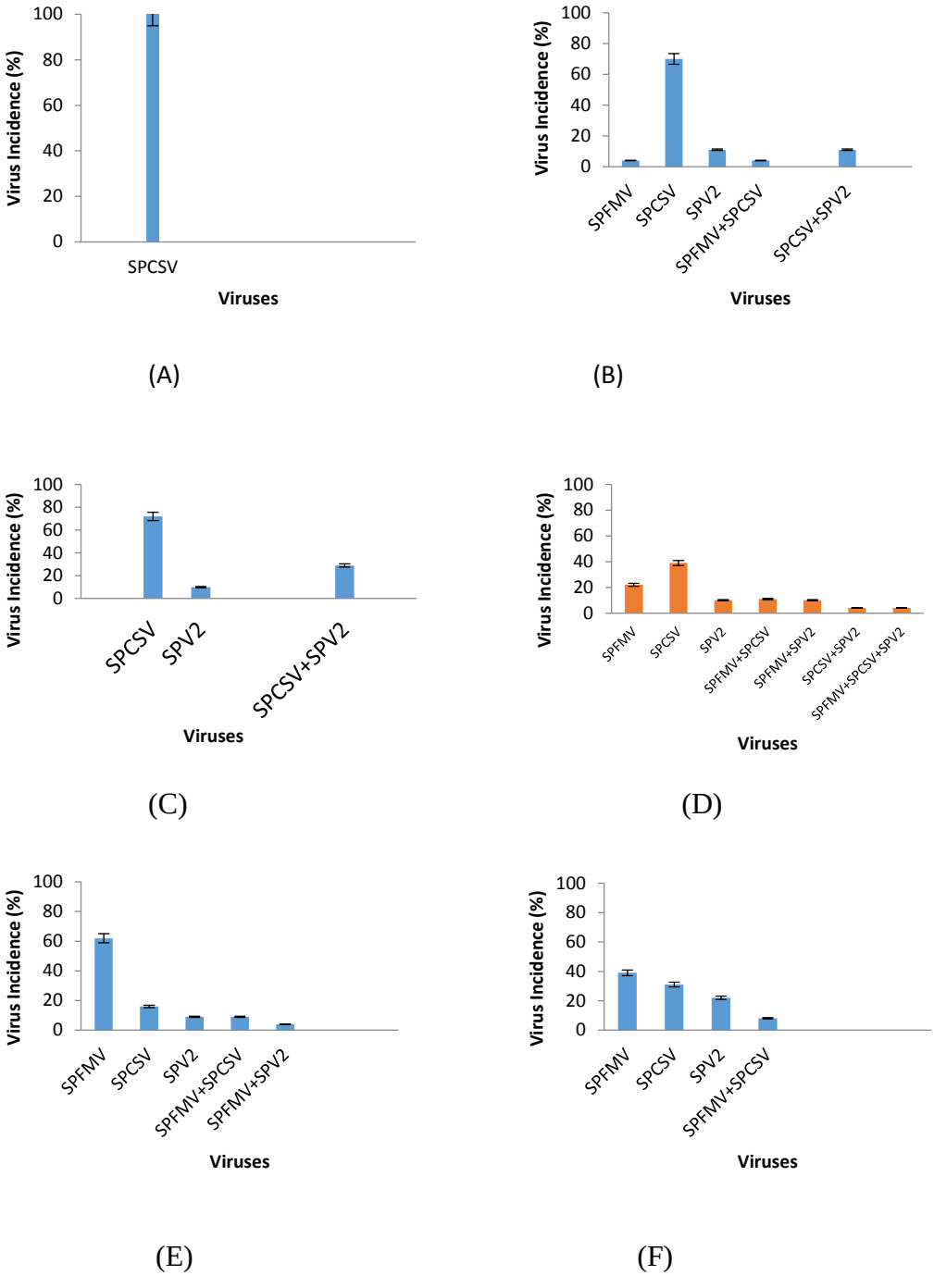
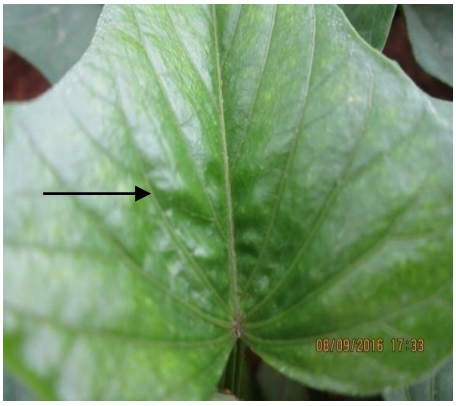


Figure 4.1: Incidence of virus diseases infecting Sweet potatoes in (A) Giwa (B) Kudan (C) Soba (D) Igabi (E) Kachia and (F) Zangon-Kataf Local Government Areas of Kaduna State during the 2016 wet season.

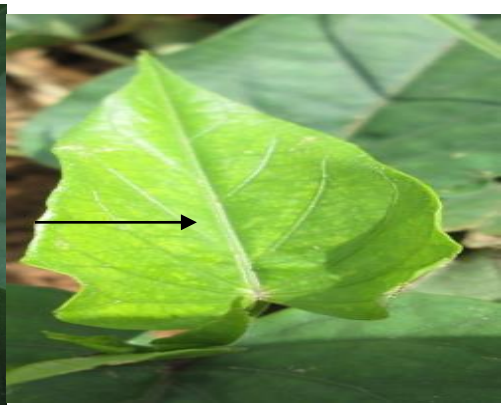
PLATE 1

A



B

C



D

PLATE 1: Infected sweet potato leaves obtained from various fields in Kaduna State. Healthy sweet potato leaf (A). Diffuse chlorotic spots and purpling induced by SPCSV (B). Severe leaf distortion,vein clearing and leaf crinkled elicited by SPVD (C). Irregular chlorotic spots, diffuse mottlings, interveinal chlorosis due to infection by SPFMV (D).

4.2 Distribution of the viruses infecting Sweet potatoes in Kaduna State

The distribution of the viruses infecting sweet potatoes in Kaduna State varied from one Local Government Area to another. SPCSV was the most prevalent virus infecting sweet potatoes. It was found in all the six local governments farms visited in the State as shown in (Figure 4.3 and 4.4). SPFMV and SPV2 were not detected at Giwa Local Government. SPCSV, SPFMV and SPV2 were detected in all the farms visited in Kudan. In Soba, SPFMV was not detected but SPCSV and SPV2 were present. In Igabi, Zangon-Kataf, and Kachia all the three viruses were detected. The triple infections of the three viruses were detected at Fanguruzan in Igabi LGA of Kaduna State (Table 4. 2).

Table 4. 2 Distribution of *Sweet potato feathery mottle virus, Sweet potato chlorotic stunt virus* and *Sweet potato virus* 2 in Giwa, Kudan, Soba, Igabi, Kachia, and Zango-Kataf Local Government Areas of Kaduna State during the 2016 wet Season

Locations Per LGA	Number of samples tested	SPFMV (%)	SPCSV (%)	SPV2 (%)	Viruses detected SPFMV+SPCSV (%)	(%) SPFMV+SPV2 (%)	SPCSV+ SPV2 (%)	SPFMV+SPCSV+SPV2 (%)
GIWA								
Sabon-Gida	7	0	3	0	0	0	0	0
Hayin-Safiu	7	0	4	0	0	0	0	0
Halkama	7	0	3	0	0	0	0	0
KUDAN								
Jaja	7	1	7	2	1	0	2	0
Angwan- Sako	7	0	5	1	0	0	1	0
Dumiga	7	0	6	0	0	0	0	0
SOBA								
Farin-Kasa	7	0	1	0	0	0	0	0
Tabasariki	7	0	2	1	1	0	1	0
Sambirini	7	0	5	0	0	0	0	0
IGABI								
Lambakau	7	3	4	2	2	1	2	0
Fanguruzan	7	2	2	0	1	0	0	1
Tumbau	7	1	4	1	0	0	1	0
KACHIA								
Angwa- Ayuba	7	5	4	0	2	0	0	0
Sakwai	7	3	0	2	0	1	0	0
Gwame	7	7	0	0	0	0	0	0
ZANGON KATAF								
Lenak	7	3	0	1	0	0	0	0
Zonkwa	7	2	3	0	1	0	0	0
Samaru- Kataf	7	0	1	2	0	0	0	0
TOTAL	126	27(21)	54(43)	12(10)	8(6)	2(2)	7(6)	1

SPFMV = *Sweet potato feathery mottle virus*, **SPCSV** = *Sweet potato chlorotic stunt virus* and **SPV2** = *Sweet potato virus*.

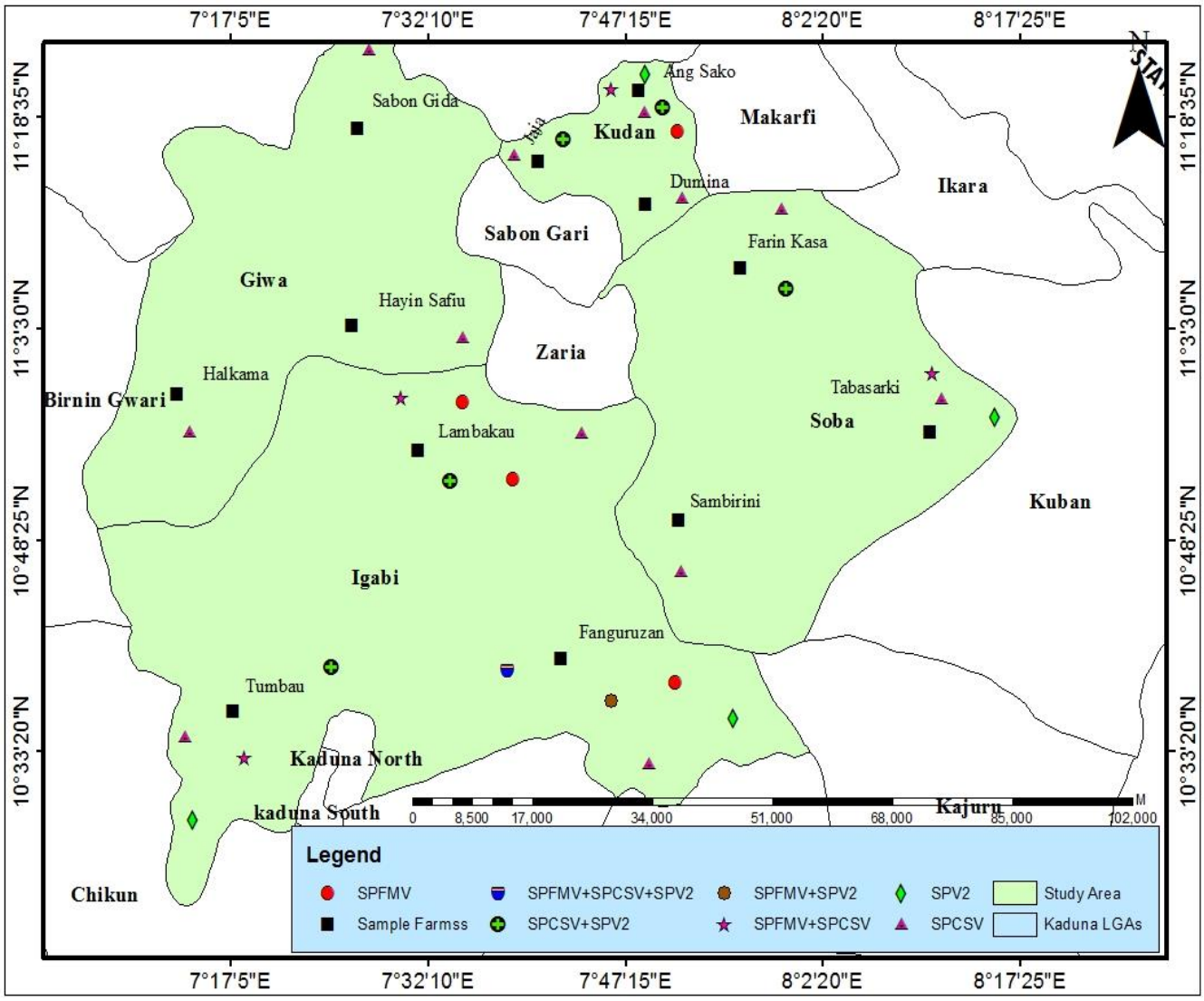


Figure 4. 2: Map of Kaduna State showing Giwa (Sabon-Gida, Hayin-Safiu and Halkama), Kudan (Jaja, Angwan-Sako and Dumiga), Soba (Farin-Kasa, Tabasarki and Sambirni), and Igabi (Lambakau, Fanguruzan and Tumbau) Local Government Areas and the distribution of the viruses detected

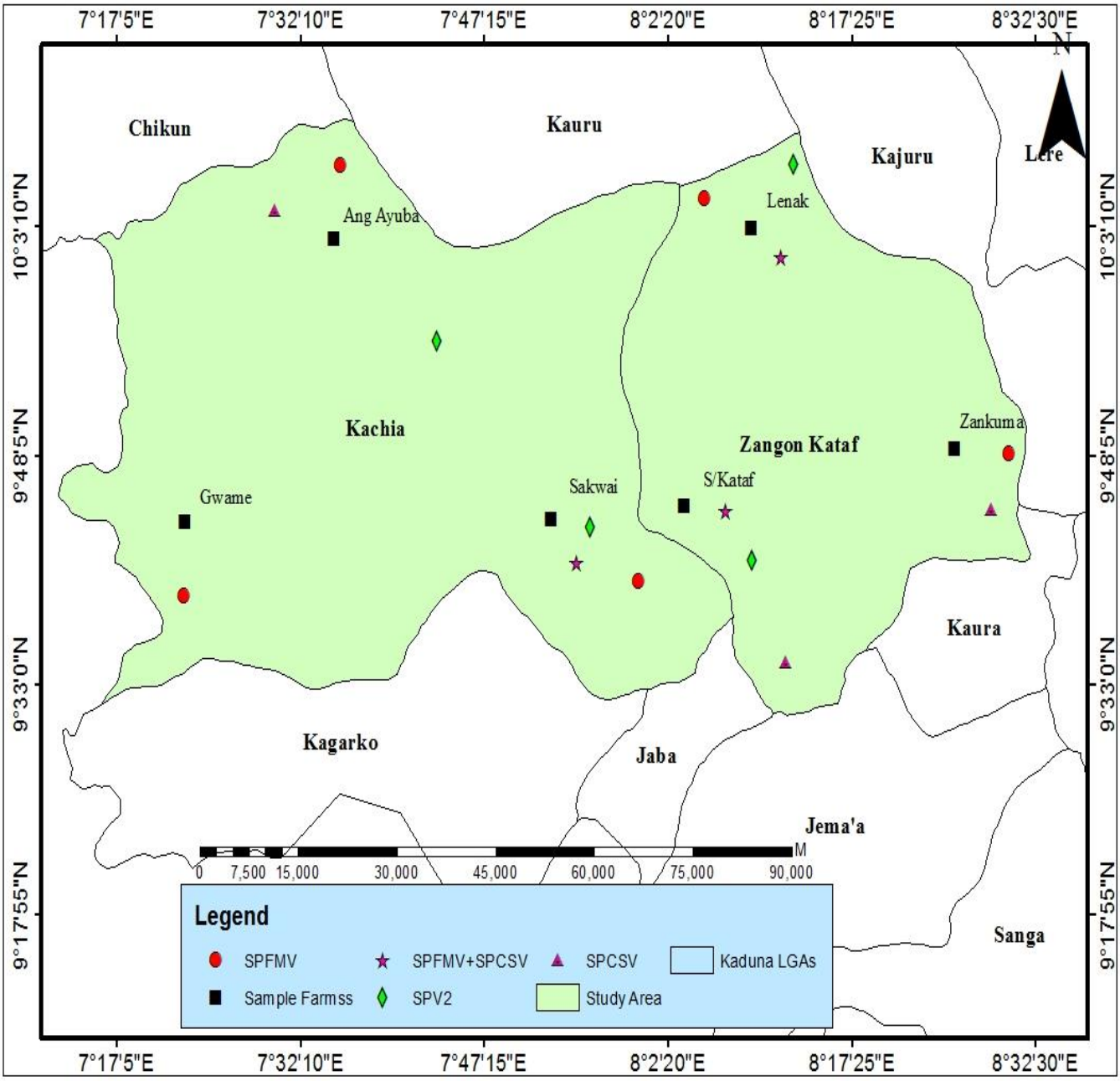


Figure 4.3: Map of Kaduna State showing Kachia (Angwanayuba, Sakwai and Gwame) and Zangon-Kataf (Lenak, Zonkwa and Samaru-Kataf) Local Government Areas and the distribution of the Viruses detected

4.3 Weed Hosts of Viruses Infecting Sweet Potatoes in Kaduna State during the 2016 Wet and 2017 Dry Seasons.

A total of one hundred and eight (108) weed samples were collected in both seasons. Fifty four (54) weed samples were collected in the 2016 wet season, and 54 as well in 2017 dry season. They were identified and found to belong to (15) plant families. In the wet season survey, the weeds that tested positive for SPFMV were identified as (*Neptunia oleraceae* Lour, *Gomphrena sessilis* Linn. DC and Morning glory *(Ipomoea hederacea L.)* family Convolvulaceae. Two weed samples (*Ageratum conyzoides* Limm and *Sclerocarpus africanus* Jacq.) from Giwa tested positive to SPV2 and three weed samples (*Ludwigia decurrens* Watt, *Sida garckeana* Polak and *Mitracarpus villosus* Sw DC.) from Kachia tested positive to SPCSV (Table 4. 3).

The ELISA results for the dry season survey showed that *Gomphrena celosioides* Von Martius, family Amaranthaceae and *Ipomoea eriocarpa* R. Brown, family convolvulaceae tested positive to SPV2 and *Chrysanthellum indicum* L., *Alternanthera pungens* Kunth both in the family Amaranthaceae and Morning glory *(Ipomoea hederacea* L.*)* family convolvulaceae were positive to SPFMV. None of the weeds tested positive to SPCSV (Table 4.3).

Table 4. 3: Weed species tested in the wet and dry season against antisera of Sweet potato viruses in Kaduna State.

			Wet season 2016			Dry season 2017	
Families of weeds tested	Scientific names of weeds	*SPCSV*	*SPFMV*	*SPV2*	*SPCSV*	*SPFMV*	*SPV2*
Acanthaceae	*Monechma ciliatum*	-	-	-	-	-	-
Amaranthaceae	*Amaranthus spinosus*	-	NF	-	-	-	-
Asteraceae	*Chrysanthellum indicum*	-	-	-	-	+	-
Lamiaceae	*Cinerea vernonia*	-	-	NF	-	NF	-
	Leucas martinicens	-	-	-	-	-	-
Rubiaceae	*Mitracarpus villosus*	-	NF	-	-	-	-
	Spermacoce verticillata	-	-	-	-	-	-
Poaceae	*Dactyloctenium aegyptium*	_	-	-	-	NF	-
Onagraceae	*Ludwiga decurens*	+	NF	-	-	-	-
Asteraceae	*Biden pilosas*	-	-	NF	-	-	-
Convolvulaceae	*Ipomoea eriocarpa*	-	NF	NF	-	-	+
Convolvulaceae	*Ipomoea hederacea* L.	-	+	-	-	+	-
Euphorbiaceae	*Euphorbia hirta* L	-	NF	-	NF	-	-
Lamiaceae	*Hyptis lanceolata*	-	-	-	-	-	-
Malvaaceae	*Malvastrum coromandelianum*	-	NF	-	-	NF	-
Amaranthaaceae	*Alternanthera Pungens*	-	-	-	-	+	-
Leguminoceae	*Senna obtusifolia*	-	-	-	-	-	-
Cyperaceae	*Kylinga pumila* Michaux	-	-	-	-	-	-
	Cyperus rotundus Linn.	-	-	-	-	-	-
	Cyperus sp.	-	-	-	-	-	-

Amaranthaceae	*Gomphrena celosioides*	-	-	-	-	-	+
Moraceae	*Neptunia oleraceae*	-	+	-	NF	-	NF
Euphorbiaceae	*Gomphrena sessilis*	-	+	-	-	-	-
Amaranthacea	*Chrysanthellum indicun*	-	-	+	-	-	-
Acanthaceae	*Alternanthera pungens*	-	-	+	-	-	-
Salviniaceae	*Ageratum conyzoides* L.	-	-	NF	-	-	-
Scrophulariaceae	*Sclerocarpus africanus*	+	-	-	-	NF	-
Mimosaceae	*Sida garckeana Mitracarpus villosus*	+	-	NF	-	NF	-
Poaceae	*Dactyloctenium aegyptium* (L.) Willd	-	-	-	-	-	-
	Pennisetum purpureum Schumach	-	-	-	-	-	-
	Acrocera zizanioides Dandy	-	-	-	-	-	-
	Eleusine indica Gaertn	-	-	-	-	-	-
	*Digitaria exilis (*Kippist) Stapf	-	-	-	-	-	-

SPCSV= *Sweet potato chlorotic stunt virus*, SPFMV = *Sweet potato feathery mottle virus*, SPV2=*Sweet potato virus* 2, + = detection, - = no detection, NF= not found.

CHAPTER FIVE

5.0 DISCUSSION

The study conducted on the incidence, distribution and alternative hosts of viruses infecting sweet potato (*Ipomoea batatas* L.) in Kaduna State of Nigeria indicated the presence of *Sweet potato feathery mottle virus* (SPFMV), *Sweet potato chlorotic stunt virus* (SPCSV) and *Sweet potato virus* (SPV2). The three viruses were detected in sweet potato plants collected during the survey in Kaduna State. The symptoms observed on sweet potato leaves appeared as faint irregular chlorotic spots, mosaic, vein clearing and leaf deformation. Similar symptoms were described in previous studies for SPFMV (Winter *et al*., 1992). There were also severe virus associated symptoms like diffuse chlorotic spots and purpling. These symptoms had previously been described in previous studies for SPCSV (Gibson and Aritua, 2002). The laboratory test conducted on these viruses confirmed that the symptoms observed were induced by the viruses. More than 14 virus diseases of sweet potatoes have been reported Moyer and Salazar, 1990; Brunt *et al.*, 1996. SPFMV and SPCSV that were identified in this study have been detected in Ibadan Nigeria (Schaefers and Terry, 1976).While SPV2 have not been reported in Nigeria to the best of my knowledge, but had been reported in research stations in Ethiopia (Adane Abrahme *et al.,* 2007).

From the results, it was confirmed that SPCSV was detected in Giwa, Kudan, Soba, Igabi, Kachia, and Zangon-Kataf Local Government Areas. The high infection rate of SPCSV compared to SPFMV and SPV2 can probably be ascribed to its dependence on transmission by whiteflies *Bemisia tabaci* Genn. which were very abundant in distribution in the farms sampled during the survey. SPFMV was detected in Kudan, Igabi, Kachia and Zangon-Kataf Local Government Areas. Karyeija *et al.* (1998) reported that this virus is transmitted non -

persistently by Aphids (*Myzus persicae* Sulzer and *Aphis gossypii* Glover) which was found on the farms visited in this locations. The farmers also confirmed they source their vines of sweet potatoes from previous harvest. This is in line with previous report by (Cadena and Campbell, 1981) who reported that the virus is seed- borne and disseminated in tubers and vines. The mixed infections of some of the virus diseases obtained in some LGAs might be due to use of vines from previous harvest which might be infected by these viruses, irregular rouging and weeding observed on almost all the farms visited during the survey which is in line with report from Gibson *et al*., (2004) who reported that the sweet potato plant and weeds serve as reservoir for these viruses. Members of the families *Convolvulaceae, Fabaceae, Euphorbiaceae, Asteraceae, Malvaceae, Solanaceae, Amaranthaceae, Chenopodiaceae, Lamiaceae, Rubiaceae and Onagraceae* were reported to be major hosts of sweet potato viruses (Cohen *et al*., 1992; Aritua *et al*., 2002; Ateka *et al*., 2002).

Sweet potato chlorotic stunt virus genus: *crinivirus* family: *criniviridеae* was detected in sweet potatoes at all the six Local Government Areas visited but was found to be highest in Giwa LGA. This virus was first reported in East Africa by Sheffield, (1957). It was also reported in International Institute of Tropical Agriculture (IITA) Ibadan Nigeria Schaefers and Terry (1976) and later by winter *et al*., (1992). This is the first report of SPCSV in northern Nigeria. In this study, it was detected most frequently in mixed infections with SPFMV than alone which is in line with previous reports (Schaefers and Terry, 1976; winter *et al*., 1992) confirming that it is a component of SPVD in Nigeria. In Nigeria, SPVD has been reported to result from the synergistic effects of the whitefly-borne virus SPSCV and aphid transmitted SPFMV (Schaefers and Terry, 1976; Clark and Moyer, 1988; Winter *et al*., 1992), and SPVD is acquired readily only from sweet potato infected with both viruses (Schaefers and Terry, 1976). Studies have

established that SPCSV provides the synergistic multiplication for SPFMV (Karyeija *et al.*, 2000b).This virus was also detected in mixed infection with SPV2 in some infected sweet potato plants. SPVD was first reported in DR Congo in (1939) and was first described in East Africa by (Sheffield, 1957).

These two viruses distorted, stunted, caused chlorosis and narrowing of leaves leading to photosynthetic disturbance. SPCSV is the more problematic component of SPVD, because yield losses due to SPFMV without SPCSV infection are relatively low and resistance of SPFMV in sweet potato breaks down after the plant is infected by SPCSV (Karyeija *et al.,* 1998; Carey *et al*., 1999; Gibson and Aritua, 2000).

Sweet potato feathery mottle virus was identified in this study in some LGAs. It was the second most prevalent virus detected. It was reported in Nigeria by Schaefers and Terry (1976) and in many African countries such as South Africa (Clark and Moyer 1988). It was detected in moderate to high incidences in Igabi, Zangon-Kataf, Kachia, and Kudan Local Government Areas, of Kaduna State. This could be attributed to the presence of Aphids (Cohen, 1997) which was observed in very large number in almost all the farms visited. This might be responsible for the transmission of the virus. Also, this virus can either be seed-borne or confined inside the tubers and vines (Sheffield, 1957) from previous harvest of which the farmers might not be aware of which might have been responsible for the incidence. This confirms the report by Zhang *et al*., (2010) that potyvirus are of the most economically important groups of plant viruses that pose a great threat to crops worldwide. Moyer and Salazar, 1989 also reported that SPFMV infect crops worldwide. SPFMV have been reported to infect some isolates of *Chenopodium amaranticolor* Coste and Reyn, *C. quinoa* Wild or *Nicotiana bentamiana* Grey

but some are restricted to the *Ipomoea* species (Thompson and Mynhardt, 1986; Clark and Moyer, 1988; Karyeija *et al*., 1998).

Sweet potato virus 2 was detected in five LGAs of the State which signifies the incidence and distribution of this virus. SPV2 was reported in research stations in Ethiopia (Adane Abrahme *et al.,* 2007), Egypt, South Africa, Kenya and other countries in Eastern Africa (Ishak *et al*., 2003; Kokkinos and Clark, 2006).The detection of this virus in some of the farms sampled and surveyed indicate the incidence and distribution of this virus in Nigeria.

Mixed infections between the viruses was also confirmed in this study. The most frequently encountered was between SPFMV and SPCSV which is the cause of SPVD. The co-infection of these viruses may be due to mixed transmission of these two viruses by their common whitefly (*Bemisia tabaci*) (Clark and Moyer, 1988; Ateka *et al*., 2005; Valverde *et al*., 2004). Sweet potato virus disease (SPVD) caused by dual infection of sweet potatoes with SPFMV and SPCSV. This research agree with previous reports from Kenya (Nyaboga *et al*., 2008), Uganda (Mukasa *et al*., 2003), Tanzania (Ndunguru and Kapinga, 2007; Ndunguru *et al*., 2009) and Rwanda (Njeruet *et al*., 2008).

Several species of weeds and other crops were observed on sweet potato fields and surrounding farms. In these findings it was found that weeds could act as source of inoculum to some sweet potato viruses. Majority of the farms visited were either mono cropped or mixed cropped with different crop plants as shown in Appendix III which probably contributed to high incidence of the virus.

The alternative host detected for SPFMV is the weed Morning glory *Ipomoea hederaceae* L. family convolvulaceae because it was carrying the virus in both seasons which is in line with

previous report form Clark *et al*., (1986) who reported that SPFMV also occurred in wild Ipomoea species which act as a reservoir. This is the first report of *Ipomoea hederaceae* L. as a reservoir host of SPFMV detected from Kaduna State.

CHAPTER SIX

6.0 SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 Summary

Field surveys were conducted during the 2016 wet season n in six Local Government Areas of Kaduna State of Nigeria to determine the presence of two important potyviruses, SPFMV and SPV2, and also SPCSV crinivirus. In January 2017 dry season, a second survey was conducted on weed plants to determine the alternative hosts of these viruses. 3 farms were visited per Local Government making a total of 18 sweet potato farms that were surveyed. A total of 126 sweet potato leave samples. 108 weed plants samples were collected in both wet and dry seasons. The virus disease incidence was calculated in each field, then per Local Government Area. Serological method, Enzyme-Linked Immunosorbent Assay (ELISA) was employed in the detection of the viruses from the samples collected. After the analyses, all the three viruses' Sweet potato feathery mottle virus, Sweet potato chlorotic stunt virus and Sweet potato virus 2 were detected. *Sweet potato chlorotic stunt virus* was detected in all the six LGAs both in single and mixed infections. *Sweet potato feathery mottle virus* was detected in four LGAs in single and mixed infections and *Sweet potato virus* 2 was detected in five LGAs in single and mixed infections. A triple infection of SPFMV, SPCSV and SPV2 were detected in Fanguruzan Igabi LGA of Kaduna State Nigeria. One weed species out of 108 tested, *Ipomoea hederaceae* L. family: Convolvulaceae was detected as reservoir host of SPFMV.

6.2 Conclusions

The following conclusions can be drawn from this research:

1) *Sweet potato chlorotic stunt virus* SPCSV (54.6%), *Sweet potato feathery mottle virus* SPFMV (21.2%) and *Sweet potato virus* SPV2 (10.3%) were found infecting sweet potatoes in Kaduna State Nigeria.
2) *Sweet potato chlorotic stunt virus* (SPCSV) was detected in all the six LGAs of Kaduna State, *Sweet potato feathery mottle virus* (SPFMV) was detected in four LGAs and Sweet *potato virus 2* was detected in five LGAs. They were detected in single and mixed infections for the first time in Kaduna State.
3) Triple infections of SPFMV, SPCSV and SPV2 was detected in Fanguruzan, at Igabi LGA of Kaduna State Nigeria.
4) *Ipomoea hederaceae* L. was detected as the reservoir host of SPFMV.

6.3 Recommendations

Based on the findings of this study, the following recommendations are suggested

1. Study of the economic impact of virus disease caused by these viruses in Nigeria is necessary and other weed hosts which serve as reservoir should be conducted in the study area and other parts of the country.
2. Biological and molecular characterization of these viruses detected will be required for better understanding of the viruses.
3. Extension agents should enlighten the farmers on the use of virus free vines.
4. Also, screening of locally acquired sweet potatoes varieties for resistance to these identified viruses.

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Appendix I: Questionnaires

Questionnaires administered to farmers in all the local government areas visited in Kaduna State Nigeria. Objective: Occurrence and distribution of viruses infecting sweet potatoes in Kaduna State.

Section A: Background information of the respondent (farmer).

STATE: ……LGA: ……VILLAGE: ……..

Gender of Farmer: Male ( ) Female ( )

Age of Farmer ……

Highest Educational Cert. (A) Non-formal (B) Primary (C) Post-primary

1.What is the size of your sweet potato farm?.....

2. For what purpose do you grow your sweet potato (A) For consumption (B) For Market (C) For both State approximately how long you have you been cultivating sweet potato …….

3. Which type of land preparation do you employ for cultivation of sweet potato?

(A) Flats (B) ridges (C) Raised basins (D) Sunken basins

4. Do you treat your vine cuttings before planting? (Yes) (No)

5. If yes, what type of treatment do you use before planting your vines?

(A) Pesticide (B) sun-heating (C) ash (D) salt soak (E) others (specify)

6. Do you grow sweet potato on the field as (A) sole crop (B) mixed crop

If yes as mixed crop, name the other crops ----------

7. If as sole crop, do you practice crop rotation (A) Yes (B) No?

8. If yes, what other crops do you rotate sweet potato with? ……

9. What length of crop rotation do you practice? (A) 2-3 (B) 3-5 (C) above5 years

10. What type of fertilizers do you apply on sweet potato? (A) Inorganic (B) organic (C) both

11. What type of crops are planted surrounding your farm ----------?

12. What type of crops protection practices do you employ in your farm----------?

13. What type of sanitary measures do you practice……?

14. What is the age of your crop? (A) ≤ 4 weeks (B) 5-9 weeks (C) ≥ 9weeks

15. Where do you get your vines? (A) Market (B) Seed company (C) From previous season

16. What type of variety do you plant? (A) Improved (B) local (C) both

Appendix II: Preparation of buffers

Coating buffer (pH 9.6)

1.59g sodium carbonate (Na_2CO_3), 2.93g sodium bicarbonate ($NaHCO_3$), 0.20g sodium azide (NaN_3) will be dissolved in 900ml H_20,adjust pH to 9.6 with HCl and make up to 1litre.

PBS (pH 7.4) phosphate buffered saline

8.0 g of sodium chloride (NaCl), 0.2g potassium chloride (KCl), 0.2g monobasic potassium phosphate (KH_2PO_4), 1.15 g dibasic sodium phosphate (Na_2HPO_4), 0.2g sodium azide (NaN_3) will be dissolved in 900ml water, adjust pH to 7.4 with NaOH or HCL and make up to 1l.

PBS-Tween (PBST)

PBS +0-5 ml Tween 20 per litre

Sample extraction buffer (pH 8.5)

0.05 M Tris containing 0.06 M sodium sulphate, pH 8.5

Conjugate buffer

PBST +2% PVP +0.2 % egg albumin

Substrate buffer

97 ml diethanolamine, 600 ml H_20, 0.2 g sodium azide (NaN_3), pH will be adjusted to 9.8 with HCl and make up to 1l with H_20.

Appendix III: Disease incidences, symptoms and cropping information of the locations surveyed in Kaduna State during the 2016 wet season

LGA	Location	No. of plants tested/field	Farm size(ha)	Virus disease incidence (%)	Insects observed	Sanitary condition	Surrounding crops	Stage of crop growth	Cropping pattern
Giwa	Sabon gida	30	0.31244	22.6	Whiteflies,Aphids.	Weeded	Sorghum, soybean and maize	Flowering	Sweet potato ,maize(I)
	Hayin safiu	30	0.27777	19.5	Whiteflies, Grasshoppers.	Weeded	Maize and sorghum	Vegetative	Sole cropping
	halkama	30	0.12226	0.0	Weevils, whitefly.	Weedy	Sorghum, tomato	Flowering	Sweet potato ,maize(I)
Kudan	jaja	30	0.21	24.1	Aphid,whitefly.	Weedy	Cowpea, millet	Flowering	Sole cropping
	Angwasako	30	14.34	15.4	Aphid,whitefly,grasshopper.	Weeded	Sorghum, cowpea,	vegetative	Sole cropping
	Dumiga	30	0.16	21.1	Aphid,butterflies,grasshopper.	Weedy	Sorghum, cowpea	Flowering	Sole cropping
Soba	Farinkasa	30	0.04	18.0	Aphids, whitefly.	Weedy	Groundnut, sorghum	Vegetative	Sweet potato maize (I)
	Tabasariki	30	0.28	24.4	Weevils, grasshoppers.	Weedy	Sorghum	Vegetative	Sole cropping
	Sambirini	30	0.15	23.5	Aphid/whitefly.	Weeded	Groundnut, sorghum	Vegetative	Sweet potato maize, groundnut (I)
Igabi	Lambakau	30	0.28	11.7	Whiteflies, grasshoppers.	Weedy	Sorghum, cowpea	Vegetative	Sweet potato yam(I)
	Fanguruzan	30	0.11	44.1	Whiteflies, grasshoppers.	Weedy	Maize	Vegetative	Sweet potato+ cowpea(I)
	tumbau	30	1.68	30.5	Aphids,ants.	Weedy	Sorghum. Millet	Vegetative	Sweetpotato+yam+okro(I)
Kachia	Angwayuba	30	0.12	22.0	Aphids,black ants.	Weedy	Ginger Groundnuts.	Vegetative	Sweetpotato,cowpea,ginger (I).
	Sakwai	30	0.32	31.2	Whiteflies, Aphids.	Weedy	Ginger, millet.	Vegetative	Sweetpotato, cowpea,yam (I).
	Gwame	30	0.22	22.2	Whiteflies, Aphids and Black ants	Weedy	Ginger.	Vegetative	Sweetpotato, cowpea,okro(I).
Zango-kataf	lenak	30	0.13	58.2	Weevils,whitefly,ants.	Weedy	Rice, sorghum	Vegetative	Sweetpotato ginger+millet(I)
	zonkwa	30	0.23	55.6	Aphids, beetles ant.	Weeded	Rice, sorghum	Vegetative	Sweetpotato+ginger okro,(I)
	Samara kataf	30	0.17	47.5	Grasshopper, Aphids	Weeded	Maize	Vegetative	Sweetpotato+cowpea ginger(I)

I= Intercropping