SERO-DETECTION AND DISTRIBUTION OF MAIZE LETHAL NECROSIS DISEASE-INDUCING VIRUSES ON MAIZE (*Zea mays* L.) AND THEIR WEED HOSTS IN KADUNA AND KANO STATES, NIGERIA

BY

Isah Abdulazeez BELLO,

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DECLARATION

I declare that the work in this dissertation entitled "Sero-detection and Distribution of Maize Lethal Necrosis Disease-inducing Viruses on Maize (*Zea mays* L.) and their Weed Hosts in Kaduna and Kano States, Nigeria" was carried out by me in the Department of Crop Protection, Faculty of Agriculture, Ahmadu Bello University, Zaria. 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 at this or any other Institution.

Bello Isah Abdulazeez _____ _____

(Student) Signature Date

CERTIFICATION

This dissertation entitled "SERO-DETECTION AND DISTRIBUTION OF MAIZE LETHAL NECROSIS DISEASE-INDUCING VIRUSES ON MAIZE (*Zea mays* L.) AND THEIR WEED HOSTS IN KADUNA AND KANO STATES, NIGERIA" by Isah Abdulazeez BELLO meets the regulations governing the award of the degree of Master of Science in Crop Protection of the Ahmadu Bello University, and is approved for its' contribution to knowledge and literary presentation.

Prof. B.D. Kashina _____ _____

(Chairman, Supervisory Committee) Signature Date

Prof. M.D. Alegbejo _____ _____

(Member, Supervisory Committee) Signature Date

Prof. O.O Banwo _____ _____

(Member, Supervisory Committee) Signature Date

Prof. O.O Banwo _____ _____

(Head of Department) Signature Date

Prof. Sani A. Abdullahi _____ _____

(Dean, School of Postgraduate Studies) Signature Date

DEDICATION

This Dissertation is dedicated to all the good people in my life.

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All praise is due to Almighty Allah, who out of His infinite mercy made it possible for me to complete this work.My profound gratitude and appreciation go to the Chairman of my Supervisory Committee, Professor Boniface David Kashina for his patience, understanding, guidance and constructive criticism towards the success of this work. I sincerely appreciate the immense attention and supervision given to me by the renowned virologists Professors Mathew David Alegbejo and Olalekan Oyeleke Banwo in spite of their tight schedules. May the Almighty reward you for such selfless sacrifice. I am very grateful to the Department of Crop Protectionfor providing me with laboratories, laboratory equipment and chemicals for my work,I appreciate the entire staff of the Department, particularly Messrs AbdulmalikZubair, Seidi Jonathan and Mrs. Hannatu Wakawa; for their assistance in the laboratory. To my colleagues Messrs. Muhammad Buhari, Ibrahim Bello, Peter Abraham,Mrs. Victoria Omolola Adedeji, and Mrs. Grace Onu, may the Almighty Allah reward you all abundantly.

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ABSTRACT

Maize (*Zea mays* L.) is the most important cereal crop in sub-Saharan Africa, however, yields are often not optimal, the deficit is partly due to pests and diseases among which Maize Lethal Necrosis disease (MLND) now figures importantly. Maize Lethal Necrosis disease is caused by coinfection of maize with *Maize chlorotic mottle virus* (MCMV), a Machlomovirusand one of several viruses from the *Potyviridae*, such as *Sugarcane mosaic virus*(SCMV) or*Maize dwarf mosaic virus*(MDMV), these *Potyviruses*have been confirmed in Nigeria Except MCMV, the primary virus of MLND. Field surveys were conducted during the 2018 dry (April) and wet (September) seasons in three Local Government Areas each in Kaduna and Kano States of Nigeria to determine the incidence and distribution of MLND component viruses (MCMV, MDMV and SCMV).A total of 18 maize farms were surveyed and a total of 135Maize and 90 weed samples were collected per season. The viruseswere detected using the Double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) and the Tripleantibody sandwich enzyme-linked immunosorbent assay (TAS-ELISA)singly, [*Sugarcane mosaic virus*(14.4%, 13%);*Maize dwarf mosaic virus* (10%, 1.6%);*Maize chlorotic mottle virus*(8.8%, 8.3%)]or as mixed infections[SCMV+MDMV (1.4%, 0.7%); SCMV+MCMV (1.1%, 2.5%); MCMV+SCMV (0.8%, 0.2%)] in both wet and dry seasons (weeds inclusive). Mixed infection by two viruses MCMV+SCMV was detected in Kura, Makarfi and Bagwai LGAs. SCMV and MCMV were detected singly and mixed in *Kyllinga squamulata, Cyperus rotundus,Spermacoce verticillata,*and singly in*Eleusine indica, Paspalum orbiculare, Setaria longiseta, Cynodon dactylon, Chloris pilosa, Saccharum officinarum, Dactyloctenium aegyptium, Setaria verticillata, Vernonia cineria* and *Oldenlandia herbacea*.MDMV was detected singly in *Senna obtusifolia* in Kura,SCMV was detected singly in *Gomphrena celosiode and Blumea aurita* in Makarfi and Lere LGAs respectively. The detection of mixed infections of *Maize chlorotic*

mottle virus, *Sugarcane mosaic virus* (MCMV+SCMV) and *Maize dwarf mosaic virus* (MCMV+MDMV) in Maize and weeds confirms the presence of Maize Lethal Necrosis disease in these States.

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CHAPTER ONE

1.0 INTRODUCTION

Maize (*Zea mays* L.) is one of the three leading global cereals that feed the world (Shiferaw et. al., 2011). Together with rice and wheat, dominates human diets (Ignaciuk and Mason-D'Croz, 2014). It is the third most important cereal crop after rice and wheat. It provides at least 30% of the food calories of more than 4.5 billion people in 94 developing countries. Maize is the global leading cereal in terms of production, with 1,016 million metric tonnes (MMT) produced on 184 million hectares globally. It is the most grown cereal crop in the world (USDA and WASDE, 2015), valued for its nutritional properties as food and feed, and as a source material for many industrial uses. Maize is of great importance worldwide because it is grown in more countries than any other cereal crop due to its adaptive versatility. It is perhaps, the most important cereal crop of significant economic importance in African countries followed by guinea corn and millet (Maredia*et. al*., 1998).

Maize contains about 72% starch, 10% protein, and 4% fat, supplying an energy density of 365 Kcal/100 g, (Nuss and Tanumihardjo, 2010). Maize provides many of the B vitamins and essential minerals along with fiber. World maize production in 2019 was 1,148,487,291 tons. Maize production in Nigeria stood at 11 MMT in 2019 (FAOSTAT, 2019).Diseases inflicted on this important plant species by plant viral pathogens are detrimental to the economy and threaten the food supplies around the globe, especially in regions like northern Nigeria where maize is a staple food crop.

The synergistic co-infection of *Maize chlorotic mottle virus* and some specific members of the *Potyviridae* like *Sugarcane mosaic virus*(SCMV) with *Maize dwarf mosaic virus* (MDMV) to cause Maize lethal necrosis disease (MLND) has been reported in Hawaii (Nelson *et al.*,

2011), Kenya (Wangai *et al*., 2012), Rwanda (Adams *et al*., 2014) and Congo (Lukanda *et al*., 2014). In maize lethal necrosis outbreaks, MCMV and SCMV is the most prevalent virus combination (Adams *et. al*., 2014). Weed species have been reported to play a significant role as alternative hosts of both plant viruses and their vectors (Asala *et al.,* 2014).Management of plant virus diseases is a matter of vital importance and concern to the farmers. It is well established that virus diseases in different crops cause enormous losses in terms of quantity and quality of products (Sastry and Zitter, 2014).

1.2 Justification of the Study

Maize is the most important cereal crop in sub-Saharan Africa (SSA). However, yields are often not optimal, so the region imports about 7 million tonnes per annum (28%) of the maize required to cover deficits in production (IITA, 2018). The deficit is partly a result of pest and disease burden, among which, Maize Lethal Necrosis disease (MLND) now figures importantly. Virus diseases among other biotic and abiotic factors present serious challenge to the successful production of maize in Africa. Complete maize production losses attributed to MLND have been reported (CIMMYT, 2012). MLND is caused by the synergistic effect of Maize chlorotic mottle virus (MCMV) and any potyvirus (Maize dwarf mosaic virus, Sugarcane mosaic virus, wheat streak mosaic virus etc.). The disease has the potential to devastate maize production across Africa. Since the first report in Kenya in 2011, MLND has spread to Tanzania, Uganda, Rwanda, and probably, other surrounding countries (Isabirye and Rwomushana, 2017).

Dangora *et al*. (2014) and Yahaya *et al*. (2014) reported MDMV in *Saccharum officinarum* and *Cymbogon giganteus* samples respectively in Makarfi Local Government Area of Kaduna

State. Muhammad *et al.* (2016) have also reported the incidence of MDMV and SCMV in Kano and Kaduna State respectively.

However, there was no information on the incidence and distribution of MCMV in these States. Therefore, it was necessary to establish whether MCMV is also present in these regions to determine its incidence alongside MDMV and SCMV to understand its impact in the epidemiology of MLND in maize fields. An important step for the implementation of management measures is an accurate diagnosis of a virus disease and mapping of its spatio-temporal occurrence in an area or crop (Naidu and Hughes 2003). To understand the spatio-temporal distribution of MCMV and MLND risk in Africa.Isabirye and Rwomushana (2017), developed ecological niche models using Genetic Algorithm for Rule Set Production (GARP) which is a computer program that creates ecological niche models for species. The generated models describe environmental conditions (precipitation, temperatures, elevation, etc.) under which the species should be able to maintain populations. Other inputs also include known detections of MCMV and MLND across Africa and their result implicated Nigeria as a potential spot for MCMV and MLND.

Pest and pathogen risk analysis is based on an evaluation of biological variables one of which is the ecological range of the pathogen compared to the host range in the importing country. Weed species have been reported to play a significant role as alternative hosts of both plant viruses and their vectors in the field (Alegbejo, 1996; Kashina *et al.*, 2003; Alegbejo and Banwo, 2005; Asala *et al.,* 2014) thereby, making the management of viruses and their vectors difficult. A clear understanding of the epidemiology of the viruses and their weed hosts is imperative for effective management of virus diseases.

Availability of accurate and reliable information on the occurrence of pests and diseases and the damage they cause is one of the essential requirements for the effective operation of the quarantine services. Unless such information is available, the plant quarantine or crop protection officer have no basis on which to act in regulating the importation of the crop and for a pathogen of such quarantine significance. Identification is key to avoid any future epidemic and one method to detect and diagnose these viruses is serological test which is one of the specific and easiest methods for rapid and precise identification.

1.3 Objectives of the Study

The objectives of this study were to determine the:

1. Incidence of *Maize dwarf mosaic virus*, *Sugarcane mosaic virus* and *Maize chlorotic mottle virus* in both rain-fed and irrigated maize in Kaduna and Kano States.
2. Weed hosts of the viruses in the study areas.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Taxonomy of Maize

Maize belongs to the tribe Maydeae of the grass family Poaceae. "*Zea*" (zela) was derived from an old Greek name for a food grass. The genus *Zea* consists of four species of which *Zea mays* L. is the most economically important. The other *Zea* spp. referred to as teosintes are largely wild grasses native to Mexico and Central America (Doebley, 1990).

2.2 Origin of Maize

Maize is one of the oldest domesticated crops,the centre of origin for Maize also known as corn (*Zea mays*) has been generally agreed to be in the Mesoamerican region, now Mexico and Central America, where it was first domesticated in the southern part about 10,000 years ago (Watson and Dallwitz, 1992).

2.3 Biology of Maize

Maize is a tall, determinate, monoecious, annual plant. It produces large, narrow, opposite leaves, borne alternatively along the length of stem. Plants have staminate spikelets in long spike-like racemes that form large spreading terminal panicles (tassels) and pistillate inflorescences in the leaf axils, in which the spikelets occur in 8 to 16 rows, approximately 30cm long, on a thickened, almost woody axis (cob). The whole structure (ear) is enclosed in numerous large foliaceous bracts and a mass of long styles (silks) protrude from the tip as a mass of silky threads (Hitchcock and Chase, 1971).

The maize plant may be defined as a metabolic system whose end product, mainly starch is deposited in specialized organs called kernel, the kernel is made up of four major parts. The seed coat (pericarp), germ (embryo), the tip cap and the endosperm. The seed coat is a layer of fibre that protects the kernel from attack by microorganisms and insects. Maize is grown industrially for its resource rich kernel. Seed dispersal of individual kernel naturally does not occur as the kernel are tightly held to the ear and hence, depends solely on man for its dissemination (Stoskopf, 1985). Pollen is produced entirely in the staminate inflorescence and eggs, entirely in the pistillate inflorescence. Maize is wind pollinated and both self and cross pollination are usually possible. Shed pollen usually remains viable for 10 to 30 minutes, but can remain viable for longer durations under favourable conditions (Coe *et al*., 1988).

2.4 Climatic Requirements for Maize Cultivation

Maize crop is essentially a warm weather crop and it is grown in wide range of climatic conditions (ICAR, 2006). Maize is produced globally across temperate and tropical zones and spanning all continents. Maize grain yield is determined, in part, by kernel number at harvest which is sensitive to environmental conditions (Ying, 2000). Maize is a versatile crop grown over a range of agro-climatic zones. It is grown from 58°N to 40°S, from below sea level to altitudes higher than 3000 m above sea level with relative humidity higher than 90% and in areas with 250 mm to more than 5000 mm of rainfall per year and with a growing cycle ranging from 3 to 13 months (Pingali, 2001).

2.5 Soil Requirements

Maize can be grown in all lands in Nigeria but it performs better in a well-drained fertile loamy soil, Maize crop generally grows well over a pH range of 5.5-7.8 (Lafitte, 1993). High yields are obtained from maize planted on deep, fine-structured, well-aerated, well-drained loamy soils that are rich in organic matter. Shallow sandy or clayey soils should be avoided whenever possible as they are more affected by drought, susceptible to erosion and reduce response to fertilizer application (Adu *et al.*, 2014).

2.6 Nutritional Composition of Maize

The maize kernel, like that of other cereal grains, includes pericarp (6%), endosperm (82%) and germ (12%) (Watson, 1987). The main structural component of the endosperm is starch, a complex carbohydrate that corresponds on average to 71% of the grain and is a source of concentrated energy. Pollens and seeds are the nutritious and edible parts of maize. Seeds serve as good source of carbohydrates. Maize contains vitamin B-complex such as B1 (thiamine), B2 (niacin), B3 (riboflavin), B5 (pantothenic acid) and B6 that makes it commendable for hair, skin, digestion, heart and brain. It contains vitamins C, A and K together with large amount of beta-carotene and fair amount of selenium that helps to improve thyroid gland and play important role in proper functioning of immune system. It has higher contents of protein and fat as compared to other cereals (Breadley, 1992).

2.7 Uses of Maize

Maize is the desired staple food for over 900 million poor consumers, it is consumed in various ways in different regions, Maize is prepared and consumed in a multitude of ways which vary from region to region or from one ethnic group to the other. For instance, maize

grains are prepared by boiling or roasting as paste (‘eko’), ‘abado’, and ‘elekute’ in Nigeria and ‘kenke’ in Ghana, or as popcorn which is eaten all over West Africa.(Abdulrahman, 2006).The vegetable part is also used in making silage for ruminants while the crop residue forms are useful source of feed for cattle during dry season, the grain is a major component of poultry and pig ration (Opaluwa *et al*., 2014). It is used as raw material by agro based industries such as beverage, soap and pharmaceuticals (Aye and Mungatana, 2012). From the ancient time corn husk has been used to pacify kapha, pitta, anorexia, general debilities, emaciation and hemorrhoids. It is a potent antioxidant that guards body from harming by free radicals responsible for cellular damage and/or cancer. It has the potential to alleviate pain and possess analgesic activity as well (Owoyele *et al*., 2010).

2.8 Maize Production

Maize is produced globally across temperate and tropical zones and spanning all continents.It is the global leading cereal in terms of production, world maize production in 2019 was 1,148,487,291 tons. Maize production in Nigeria stood at 11 MMT in 2019 (FAOSTAT, 2019). Maize occupies approximately 24% of farmland in Africa and the average yield stagnates at around 2 tons/hectare/year (IITA, 2018).

2.9 Constraints to Maize Production

Two factors—biotic and abiotic, are identified among the constraints limiting maize crop production in sub-Saharan Africa. The abiotic factors include temperature, rainfall, solar radiation, soil pH. The biotic factors include pests and diseases. Abiotic factors are affected by climate change which poses significant risks to future crop productivity as temperatures rise, rainfall patterns become more variable and pest and disease pressures increase (Heisey and Rubenstein, 2015). Climate change affects the poorest populations most, in terms of food security. The number of malnourished children in sub-Saharan Africa is expected to increase as the severity of climate change increases. Climate change models have suggested that average maize yields are likely to fall between 5 and 33% by 2050, depending on the severity of climate change (Nelson and Rosegrant, 2010), with the largest decrease in productivity occurring in the least developed countries (Ignaciuk, 2014). As a result of climate change, maize prices could increase by up to 30% (Ignaciuk and Mason-D'Croz 2014). Climate limits the production area of maize and lack of rainfall (drought) or too much of it (flood) can result in 100% loss of maize output (Chi-chung and Mccarl, 2004). Several studies reported that availability of adequate rainfall is by far the most limiting factor in maize production in sub-Saharan Africa (CIMMYT, 1988; Diallo *et al.*, 1989). Intermittent drought has been implicated among the major constraints limiting the production of maize in the Guinea Savanna of West Africa (Kamara *et al.*, 2005).

The effect of soil pH outside the range of 5.5 – 7.8 usually makes certain elements more or less available, so toxicity or deficiency develops. Below a soil pH of 5.5, Al and Mn toxicities are frequently problems, and deficiencies of P and Mg are common. At a pH above 8 (or above 7 on calcareous soils), deficiencies of Fe, Mn, and Zn tend to occur. The field

symptoms of unsuitable pH thus generally resemble those of micronutrient problem.Soil fertility is among the major constraints limiting the production of maize in the Guinea Savanna of West Africa (Kamara *et al.*, 2005).The biotic factors that limit maize production are also diverse, some of the most important being insects and closely related organisms such as mites. From the high valleys of the tropics and subtropics to the tropical lowlands and temperate regions, these pests can infest maize at any stage of crop development and in storageattacking any part of the plant, often causing severe damage or transmitting diseases (Ortega, 1987).

Various species of stem borers rank as the most devastating maize insect pests in sub-Saharan Africa.They can cause 20-40% yield losses during cultivation and 30–90% biological postharvest losses. Other pests in SSA include ear borers, armyworms, cutworms, grain moths, beetles, weevils, grain borers, rootworms, and white grubs. The parasitic *Striga* weed is another maize pest. Yield losses due to*Striga* infestation range from 10 to 100% depending on time of infestation (Lagoke *at al.*,1991). Weed-related yield losses ranging from 65 to 92% have been recorded in the Nigerian savanna (Maddonni *et al*., 2006). Okiwelu *et al*. (1987) recorded high level of moisture, combined with a decrease in germination ability of maize due to infestation by *Sitophilus zeamais*, in Nigeria.

Maize in Nigeria is attacked by an array of diseases that can cause significant losses, these include the Downey mildew, rust, leaf blight, ear rots, leaf spots and *Maize streak virus* (IITA, 2009). Some nematodes of maize are *Meloidogyne* and*Heterodera* spp. (Nicol*et al.*, 2011), and*hoplolaimus* and *Pratylenchus* species (Keetch, 1989).

2.9.1 Viruses of Maize

Some of the viruses of maize in tropical Africaare *Maize streak virus* (MSV), *Maize mottle/chlorotic stunt virus* (MMCSV), *Maize stripe virus* (MStpV), *Maize dwarf mosaic virus* (MDMV), *Sugarcane mosaic virus* (SCMV), *Guinea grass mosaic virus* (GGMV)*Maize mosaic virus* (MMV) *Maize chlorotic mottle virus* (MCMV) (Thottappilly *et al*., 1993).

2.9.2*Maize chlorotic mottle virus* (MCMV)

Disease and virus were originally described by Hebert and Castillo (1973) and Castillo and Hebert (1974). *Maize chlorotic mottle virus* (MCMV) is the type and only species of the *Machlomovirus* genus.The following serotypes of MCMV have been identified: Kansas serotypes 1 and 2 and the Peru serotype (Niblett and Claflin, 1978; Uyemoto*et al*., 1980).

2.9.2.1 Host range of *Maize chlorotic mottle virus*

Maize is the only known natural host of MCMV (Bockelman *et al*., 1982), The experimental host range of the virus is restricted to the Poaceae family, namely, *Eleusine coracana* (finger millet), *Saccharum officinarum* (sugarcane), *Sorghum halepense* (Johnson grass), *Zea diploperennis Zea mays* (maize), *Zea mays subsp. mays* (sweetcorn) (Castillo and Hebert, 1974; Bockelman *et al*., 1982).

2.9.2.2 Geographical distribution of *Maize chlorotic mottle virus*

Maize chlorotic mottle virus has been reported in several countries throughout the Western Hemisphere including Argentina, Mexico, Peru and the USA. It is probably also present but not yet reported in other Western Hemisphere countries. Its vector, *Diabrotica* spp., are strictly New World species; thus, MCMV has the potential to be present and become

established in any maize-growing region within the Western Hemisphere. The report from Hawaii that MCMV can be transmitted by Thrips (*Frankliniella williamsi*), significantly increases the potential range of the virus. In Nebraska and Kansas (USA), MCMV was initially restricted to the Republican River valley which borders these two States (Uyemoto, 1983). In Kauai, Hawaii, the virus is present in winter nursery maize breeding plots (Jiang, *et al*.,1992). MCMV is endemic in the Lima, Huaral, Chancay, Lurin and Canete valleys in the Department of Lima, Peru (Castillo, 1976). It is important to note that MCMV and maize mottle chlorotic stunt virus, which is endemic to tropical Africa, are two distinct and unrelated viruses (Thottappilly *et al.*, 1993). The two taxonomically, morphologically and geographically distinct viruses should not be confused because of their similar names.

2.9.2.3 Vectors and Transmission of *Maize chlorotic mottle virus*

*Maize chlorotic mottle virus*is transmitted by six species of beetles, belonging to the family Chrysomelidae: the cereal leaf beetle (*Oulema melanopus*), the corn flea beetle (*Systena frontalis*) and *Chaetocnema pulicaria*, the southern corn rootworm (*Diabrotica undecimpunctata*), the northern corn rootworm (*D. longicornis*) and the western corn rootworm (*D. virgifera*) (Nault *et al*., 1978). In Hawaii, MCMV has been shown to be transmitted by the thrips: *Frankliniella williamsi* (Jiang *et al*., 1992).

2.9.2.4 Symptoms of *Maize chlorotic mottle virus*

For field-grown maize infected with MCMV, growth is stunted with the formation of short internodes. Leaf symptoms begin as chlorotic stripes running parallel to the veins which later coalesce to produce elongated chlorotic blotches, finally resulting in leaf necrosis and epinasty. In severe infections of particularly susceptible lines, leaf necrosis can result in plant

death (Castillo and Hebert, 1974). Male inflorescences have hard panicles, short rachis and few spikelets. Fewer ears and ear malformation can also occur in severe infections. A general observation is that the younger the maize plant is when MCMV infects, the more severe the stunting and symptoms become (Lenardon *et al*., 1985).

2.9.2.5 Transmission of *Maize chlorotic mottle virus*

Thrips were able to transmit MCMV after acquisition and inoculation access periods of 3hours. *Maize chlorotic mottle virus* is transmitted byThrips in a semi-persistent manner (Cabanas, 2013). The virus is also easily transmitted mechanically in the laboratory.

2.9.1.7 Particle structure of *Maize chlorotic mottle virus*

The virus has spherical particles 30 nm in diameter when observed in a transmission electron microscope after negative staining (Castillo and Hebert, 1974). The virions appear smooth and icosahedral in shape. From electron micrographic observations and the sequence similarity of the coat protein with that of *Southern bean mosaic*Sobemovirus, the virion is hypothesized to be a t=3 icosahedral virion formed by 180 copies of the 27 kDa capsid protein subunit. The virion packages the 4,400 nucleotide single-stranded RNA genome (Lommel *et al*., 1991).

2.9.2.6 Economic importance of *Maize chlorotic mottle virus*

In Peru, losses in floury and sweet maize varieties due to MCMV have been reported to average between 10 and 15%. In experimental plots, inoculated plant yields were reduced by up to 59% (Castillo, 1976). In Kansas crop losses due to maize lethal necrosis (caused by MCMV and any potyvirus) have been estimated to be between 50% (Uyemoto *et al*., 1980) and 90% (Niblett and Claflin, 1978), In Kenya an estimated 77,000 acres of maize were

affected by MLND in 2012, resulting in yield losses of 126 million metric tons equal to $52 million US dollars (Wangai *et al*., 2012). *Maize chlorotic mottle virus* rapidly spread to Rwanda and the Democratic Republic of the Congo(Adams *et al*., 2014; Lukanda *et al*., 2014). *Maize chlorotic mottle virus*is predicted to continue to spread to maize production regions across Africa and interact synergistically with established potyviruses to cause MLND (Isabirye and Rwomushana, 2017).

2.9.3*Maize dwarf mosaic virus*

Maize dwarf mosaic potyvirus (MDMV) was described by Williams and Alexander (1965). Maize dwarf mosaic virus belonging to the genus Potyvirus (Potyviridae), is a serious maize pathogen causing leaf mosaic and stunting in maize plants (Gordon, 2004; Fuchs, 2004).

2.9.3.1 Host range of *Maize dwarf mosaic virus*

Maize and Johnson grass (*Sorghum halepense*) (for certain strains) are natural hosts of MDMV. Sugarcane, sorghum, maize, *Eleusine* spp., *Panicum* spp. and *Setaria* spp. are hosts of SCMV (Brunt *et al.*, 1990).

2.9.3.2 Geographical distribution of *Maize dwarf mosaic virus*

Maize dwarf mosaic virus -infected maize plants were reported in maize grown at mid-to high altitudes in Rwanda during a survey in 1983 (IITA 1984). It has been reported in Nigeria (IITA, 1977) and Côte d'Ivoire (Fauquet and Thouvenel, 1987). *Sugarcane mosaic virus* is considered an important disease in the mid altitude region of East Africa(Kulkarni, 1973). It has also been reported on maize in Madagascar, Réunion, and South Africa,(Autrey 1983). MDMV has also been reported in Kaduna by (Yahaya *et al.*, 2014; Dangora *et al.*, 2014) on weed hosts and sugarcane respectively.

2.9.3.3 Vectors of *Maize dwarf mosaic virus*

The vectors include several species of aphids including *Rhopalosiphum maidis, R. padi, Myzus persicae, Schizaphis graminum* and *Aphis craccivora.* (Shukla*et al*., 1989).

The aphid species known to be efficient vectors of MDMV are: *Schizaphis graminum* (Rondani), *Aphis maidiradicis* Forbes, *A. craccivora* Koch, *A. fabae* Scopoli, *A. gossypii* Glover, *Hyalopterus atriplicis* (L.), *Acyrthosiphon pisum* (Harris), *Myzus persicae* (Sulzer), *Macrosiphum avenae* (F), *Rhopalomyzus poae* (Gillette) and *Rhopalosiphum padi* (L.) (Knoke *et al*., 1974).

2.9.3.4 Symptoms of *Maize dwarf mosaic virus*

Diseased plants show irregular chlorosis, mottle, striping, and a tendency to develop ring-like flecks. Sensitive varieties may show extreme distortion and stunted growth. The severity of disease symptoms may vary widely among plants in open-pollinated maize populations (Louie and Darrah, 1980).

2.9.3.5 Transmission of *Maize dwarf mosaic virus*

Maize dwarf mosaic virus is transmitted in a non-persistent manner by aphids. Aphids can acquire and transmit the virus in a matter of minutes (Ford *et al.*, 2004).Non-persistently transmitted viruses are traditionally perceived as only being retained by the aphid for a maximum of 1-2 hours. However, under experimental conditions, viruliferous *Schizaphis graminum* have been observed to retain infectivity for over 20 hours (Berger *et al.*, 1987).

The virus is also easily sap-transmissible, Sap transmission tests show that most species of Poaceae are susceptible, some being infected symptomlessly (Ford and Tosic, 1972; Tosicand Ford, 1974). Seed transmission has also been reported (Ford *et al*., 1989).

2.9.3.6 Particle structure of *Maize dwarf mosaic virus*

Maize dwarf mosaic virus is a member of the genus *Potyvirus* in the family *Potyviridae*. It has filamentous virions, 750 nm long × 13 nm wide, and an ssRNA genome consisting of 9515 nucleotides (Shukla *et al*., 1994). *Maize dwarf mosaic virus* is closely related to *Sugarcane mosaic virus* (SCMV), *Johnsongrass mosaic virus* (JgMV), *Sorghum mosaic virus* (SrMV) and *Zea mosaic virus* (ZeMV) and together these constitute the *Sugarcane mosaic virus* subgroup of Potyviruses. In earlier literature, there was considerable confusion in the taxonomy of the *sugarcane mosaic virus* subgroup. Initially, all potyviruses infecting maize were labelled MDMV and all Potyviruses infecting sugarcane were labelled SCMV. It is now recognized that in the USA, both SCMV and MDMV infect maize causing similar mosaic symptoms, and what was previously referred to as MDMV strain B is in fact a strain of SCMV (Shukla *et al*., 1994). Five strains of MDMV, strains A, C, D, E and F, are still recognized based on symptomatology on inbred maize lines including inbred line N20 and frequency of transmission by different aphid species (Shukla *et al*., 1994).

2.9.3.7 Economic importance and impact of *Maize dwarf mosaic virus*

Maize dwarf mosaic virus is a serious maize pathogen, epidemic worldwide, and one of the most common virus diseases for monocotyledonous plants, causing up to 70% loss in corn yield globally since 1960, MDMV is amongst the most important virus diseases in maize worldwide (Kannan, 2018).*Maize dwarf mosaic virus* causes economic losses in maize.

Infection on maize plants affects growth stages of development, fertility and mature weights, number of seeds and rows, yield (seed yield, total yield), 1000 grain weight, seed quality (viability), and susceptibility of infected plants to other pathogens/diseases. The extent of pathological changes induced by MDMV mainly depends on cultivar susceptibility, time of infection, water conditions, nutrients etc.Early infection with MDMV delays all stages of maize development, for 2 weeks or more (Scott and Nelson, 1972).

The height of maize plants infected with MDMV is reduced by up to 23% (Tosic and Misovic, 1967). In extreme cases, especially with maize inbred lines, the diseased plants are 25-50% of the height of healthy plants (Janson and Ellet, 1963; Tosic *et al*., 1990a).

Sterility, which is more frequent with infected maize plants, contributes to the economic impact of MDMV. Reduced growth and development, and changes in some plant development stages (Mikel *et al*., 1981) can lead to sterility in up to 25% of infected plants (Tosic and Misovic, 1967), but in the case of maize inbred lines the sterility can be even higher (Tosic *et al*., 1990). The total yield of maize can be very much affected by MDMV infection. Yield reduction per fertile field maize plant infected with MDMV can be up to 42% (Tosic and Misovic, 1967). However, with maize inbred lines or with sweetcorn, especially after later sowing, the yield can be reduced by 75% or more (Forster *et al*., 1980).

Germinability of seeds formed on maize plants infected with MDMV may be reduced by nearly 20%, while the length and width of primary roots are smaller by 7.4 and 20.0%, respectively (Stakic and Savic, 1984). When Janson and Ellett first reported the disease from southern Ohio, they observed nearly total losses in fields of late-planted sweet corn. Important subjects of inquiry currently attracting considerable interest and research effort are the epidemiology and genetics of resistance of maize dwarf mosaic (Knoke *et al*., (1983).

2.9.4*Sugarcane mosaic virus* (SCMV)

Sugarcane mosaic virus (SCMV) belongs to the sugarcane mosaic subgroup of the Potyviridae,togetherwith *Maize dwarf mosaic virus* (MDMV), *Johnsongrass mosaic virus* (JGMV), *Sorghum mosaic virus* (SrMV), *Zea mosaic virus* (ZMV), *Pennisetum mosaic virus* (PeMV) and *Cocksfoot strike virus* (CSV). *Sugarcane mosaic virus* is an important virus pathogen especially in European and Chinese maize production, causing huge losses in grain and forage yield in susceptible cultivars(Gan *et al*., 2010).

2.9.4.1 Host range of *Sugarcane mosaic virus*

Sugarcane mosaic virus has a wide host range, including Gramineae family and occurs in many regions of the world. The virus causes mosaic symptoms in sugarcane, maize, sorghum, and other poaceous plants (Yang and Mirkov, 2007) with maize and sugarcane being the natural hosts (Scheets, 2004).

2.9.4.2 Geographical distribution of *Sugarcane mosaic virus*

The mosaic disease of sugarcane was first noticed in Porto Rico in 1916. It spread rapidly and then was discovered in Louisiana and other southern States in the US, though the pathogen of this disease was not identified(Brandes, 1919). *Sugarcane mosaic virus*was first identified in Ohio, the US in 1963 (Janson and Ellet, 1963).

Sugarcane mosaic virus has been reported in 26 countries, namely: Nigeria, Argentina, Australia, Brazil, Cameroon, China, Colombia, Congo, Denmark, Ethiopia, Egypt, France, Germany, India, Iran, Israel, Mexico, Netherlands, Pakistan, Poland, South Africa, Spain, Thailand, the United Kingdom, the United States and Vietnam. In China, it was first observed in Henan Province in 1968.

2.9.4.3 Vectors of *Sugarcane mosaic virus*

The vectors include several species of aphids including *Rhopalosiphum maidis, R. padi*, *Myzus persicae, Schizaphis graminum*, and *Aphis craccivora.* (Shukla*et al*., 1989).

2.9.4.4 Symptomsof *Sugarcane mosaic virus*

Symptoms of Sugarcane mosaic virus include intense mottling throughout the laminar region of the plant, characterized by discoloration of the plants leaves, and growth stunting (Lockhart*et al.*, 1996).

2.9.4.4 Transmissionof *Sugarcane mosaic virus*

Transmitted in a non-persistent manner by aphids like MDMV. Bond and Pirone (1970) reported soil transmission of SCMV, seed transmission of upto 0.4% has also been reported by Shepherd and Holdeman (1965).

2.9.4.5 Economic importanceof *Sugarcane mosaic virus*

The family *Potyviridae*, which includes approximately 200 species of economically important plant viruses, causes significant losses in agricultural, pasture, horticultural and ornamental crops (Wu, *et al*., 2012). Sugarcane mosaic virus is one of the largest and most economically important plant viruses due to its wide host range. In the mid-1920s, epidemics of the disease nearly collapsed the sugarcane industry in Argentina, Brazil, Cuba, and southern United States (Croft *et al*., 1996). In Australia, losses have been reported due to SCMV causing major problems in most of the sugarcane growing countries and many varieties have gone out of cultivation due to yield losses of up to 50% (Viswanathan & Balamuralikrishnan, 2005).

2.9.5 Maize lethal necrosis disease (MLND)

Maize lethal necrosis disease is caused by a combination of MCMV with other Potyviridae family like SCMV, WSMV or MDMV (Bockelman *et al*., 1982). The double infection (co-infection) which is more severe than single infection (Niblett and Claflin, 1978; Scheets, 1998) occurs mostly with two viruses; MCMV and SCMV and this gives rise to what is known as MLND, also referred to as Corn Lethal Necrosis (CLN) (Uyemoto *et al*., 1980; Uyemoto *et al*., 1981).

2.9.5.1 Geographical distribution of MLND

In September 2011, the first outbreak of MLND was reported in east Africa along rift valley regions of Kenya (Wangai *et al*., 2012). Regions that were reported to have the disease includes; Bomet, Naivasha, Narok, Chepalungu, Sotik, Transmara, Bureti, Nakuru, Konoin, South Narok, Mathira East, Imenti South Districts and Nyeri (Wangai *et al*., 2012). In August 2012, this disease was also reported in Tanzania around border regions especially Northern zone and along Lake Zone (Makumbi and Wangai, 2013). Northern zone includes Arusha (Karatu, Mlangarini, Longijave and Ngaramtoni), Kilimanjaro (Hai district in Nshara and lower Moshi) and Manyara in Kiru, Babati, Mbulu and Simanjiro. Lake Zone includes Mwanza, Mara and Shinyanga. In Uganda, the disease was first reported in October 2012 in Busia then in border district of Tororo, Mbale and Kapchorwa (ASARECA, 2013). Maize lethal necrosis disease was first reported in February 2013 in Gisesero site, Musanze District in Northern Province of Rwanda and it then spread to western Province (Adams *et al*., 2014; ASARECA, 2013).This disease is new in east Africa but not new in the other parts of the world as it was identified as corn lethal necrosis in 1976 in Kansas (Niblett and Claflin, 1978; Uyemoto, 1983), Peru (Uyemoto, 1983), Hawaii in the early 1990s (Nelson *et al*., 2011),

Nebraska in 1976 (Uyemoto, 1983), Argentina(Gordon *et al*., 1984), Texas and Brazil (Uyemoto, 1983). The possibility of spreading to other areas cannot be ruled out and hence need to quantify its distribution in a wider context.

2.9.5.2 Symptomsof MLND

Symptoms of MLND includes; elongated yellow streaks parallel to leaf veins, streaks may coalesce to create chlorotic mottling, chlorotic mottling may be followed by leaf necrosis (Nelson *et al*., 2011; Makone *et al*., 2014) which may lead to "dead heart" symptom and plant death (Wangai *et al*., 2012), premature aging of the plants (Gordon *et al*., 1984), failure to tassel and sterility in male plants, malformed or no ears (Uyemoto *et al*., 1981; Gordon *et al*., 1984), failure of cobs to put on grains and rotting of cobs (Wangai *et al*., 2012)

2.9.5.3 Economic importanceof MLND

Maize lethal Necrosis Disease is a big threat to maize production in east Africa as it can cause intensive to complete yield loss, Maize is susceptible to this disease at all stages of development specifically from seedling stage to near maturity. It was first reported in Kenya in September 2011 in Bomet County with a yield loss of 90 – 100% (Wangai *et al*., 2012), ASARECA (2013) reported MLND to cause yield losses of 50 to 80% in Uganda. yield loss of 50.5% valued at US$ 332 per hectare has been recorded by Kagoda (2016). On the other hand, maize production costs are increasing as farmers use herbicides and insecticides to control weeds and insect vectors transmitting the disease. Furthermore, seed production costs also increaseas extra cost of seed treatment is incurred. Therefore, proper solution must be found to properly manage the MLND to reduce the losses and maximize production.

2.9.6 Weed Hosts Range for *Maize chlorotic mottle virus*, *Maize dwarf mosaic virus* and *Sugarcane mosaic virus*.

Hosts that can be infected experimentally are limited to the grasses in the family *Poaceae* (Scheets, 2004). The following genera were found to be susceptible to MCMV during experimental inoculation: Andropogon, *Avena, Bouteloua, Buchloe, Calamovilfa, Eleucine, Eragrostris, Euchlaena, Hordeum, Secale, Sorgastrum, Sorghum, Spartina, Tripascum, triticum.* There has been field report of MCMV on *Sorghum halapense*, a perennial host (Achon *et al.*, 2017). These eight genera have been reported by Rosenkranz (1980) as containing hosts of MCMV and SCMV: *Arthraxon, Brachypodium, Hyparrhenia, Leersia, Tragus, Trichachne, Trichloris* and *Vaseyochloa.* Common reed (*Phragmites communis* Trin.) has been confirmed by Ilbagi (2006)as a perennial host of MDMV and MCMV.

2.10 General Management of Maize Viruses

The most common approaches for management of virus diseases in maize are divided into three main approaches, which include:

(1) Breaking pathogen–vector and pathogen–maize interactions by removing virus reservoirs

(2) Breaking vector–maize interactions by reducing vector numbers on susceptible maize or deploying insect resistant crops; and

(3) Breaking pathogen–maize interactions by deploying virus resistant crops.

2.10.1 Removing pathogen reservoir

For maize virus diseases, vectors, weedy grasses, other poaceous crops, and maize itself can serve as reservoirs or sources of disease-causing viruses. In tropical regions such as Hawaii,

Africa, and Central America, year-round maize production can result in sequential, overlapping crops with no maize-free periods. Here, the older maize crop can serve as a virus reservoir for a newly planted crop (Shepherd *et al*., 2010). The mature maize crop also serves as a source of vectors for transmitting viruses. For seed-transmitted viruses such as the Potyviruses, WMoV, and MCMV, maize can also serve as a pathogen source (Hohmann*et al.*, 1999; Jensen *et al*., 1991; Lebas *et al.*, 2005). It is important to note that the presence of a virus in maize seed does not always mean that the virus is transmitted (Hohmann *et al*., 1999), because of the possibility for phytosanitary conditions and restrictions on maize shipment that may be implemented in an effort to control diseases caused by seed-transmitted viruses.

In temperate regions, Johnsongrass is an important perennial reservoir of MDMV and MCDV (All, 1983). Weed eradication reduced the incidence of these diseases in the USA, but required very high levels of weed control. In maize–soybean rotations, control of Johnsongrass in the soybean crop has positive effects on virus disease control in the maize crop. Because MDMV and MCDV are acquired within minutes of feeding on virus-infected weeds, using post-emergent herbicides to control Johnsongrass after maize crops have emerged can make viruliferous vectors move from dying weeds to maize and increase disease incidence (Eberwine and Hagood, 1995). Other poaceous crops and weeds can also serve as vector and virus reservoirs, with winter wheat serving as an important overwintering host for both the viruses and their vectors. Maize and wheat are frequently grown in close proximity in China and Argentina, leading to an increase in RBSDV and MRCV incidence (Li *et al.*, 1999; Grilli, 2008). In these cases, delaying the maize planting date to avoid large populations of vectors can provide some disease control. Vectors themselves can be reservoirs of propagative transmitted pathogens. Recent outbreaks of Maize rough dwarf in Europe were associated

with high *L. striatellus* populations when the maize crop was at early developmental stages. The increased vector populations were thought to be the result, at least in part, of vector migration into the region(Achon*et al*., 2013). In areas where maize is not grown in the winter, corn stunt infected *D. maidis* overwinters on other crops such as alfalfa and returns to infect a new maize crop the next spring. In fact, corn stunt infection improved vector survival (Ebbert and Nault, 1994). Here, measures that reduce vector populations can be helpful.

2.10.2 Disrupting vector-maize interactions

Treatments that decrease vector populations reduce interactions between the vectors and maize and can be useful for controlling virus diseases. For persistently and semi-persistently transmitted viruses, including MRDV, insecticide treatments directed at vector populations can reduce disease incidence (Antignus*et al.*, 2008). However, insecticide treatments are not generally effective for non-persistently transmitted viruses that are transmitted within a few minutes of insect feeding (Jones, 2006). In other cases, adjusting planting dates and crop rotations have provided some disease control by limiting the access of viruliferous vectors to crops (Fritts *et al*., 1999). Barriers, such as tall plants, can also reduce vector access to maize and provide control for diseases caused by non-persistently transmitted viruses (Jones, 2006). Another emerging area of research is to deploy vector-resistant crops, and maize inbred lines with resistance to *P. maidis*, *C. mbila*, and the aphid *Rhopalosiphum maidis* (Fitch) have been characterized (So*et al.*, 2010). The rapidly developing genome information on maize virus vectors is expected to be key to identifying targets for development of vector-resistant maize (Cassone *et al.*, 2014).

2.10.3 Maize lethal necrosis disease resistance in Maize

Deploying virus-resistant crops is considered the most economically and environmentally sustainable approach to controlling disease. Maize is a highly diverse crop, and remarkable germplasm resources are available worldwide to maize breeders and geneticists (Romay *et al*., 2013). It is therefore, not surprising that lines with moderate to strong resistance to the major maize-infecting viruses have been identified. Genetic analyses of resistant lines indicate that virus resistance in maize is primarily monogenic or oligogenic and is either dominant or quantitative in character. Major and minor genes associated with virus resistance have been mapped using a number of different approaches by taking advantage of the array of genotyping tools that have been developed (Schaeffer *et al*., 2011; Flint-Garcia, 2013).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Survey and Sampling of Maize Fields

Survey was conducted during the September 2018 wet season and April 2018 dry season to determine the occurrence of *Maize dwarf mosaic virus* (MDMV), *Maize chlorotic mottle virus* (MCMV) and *Sugarcane mosaic virus* on both rain-fed and irrigated maize fields in three Local Government Areas (LGAs) each of Kaduna and Kano States. Three farms each from highest to the lowest maize producing LGAs were surveyed per State. In each field, 3× 3 m^2 sized-quadrats was set up at four corners of the field and one at the center. Weeds within and around the maize farms were randomly sampled. The number of infected and total number of plants in the quadrat were recorded, both during the wet and dry seasons from the same fields.

Leaf samples were collected, carefully labeled, wrapped in polyethylene bags, stored in an ice chest and then transported to the Virology Laboratory of the Department of Crop Protection, Ahmadu Bello University(A.B.U), Zaria. For analysis. The samples were stored at −4 °C prior to diagnosis. Collected weed samples were identified at the Herbarium of the Department of Botany, Faculty of Life Sciences, Ahmadu Bello University, (ABU). Zaria. Disease incidence was calculated by dividing the number of diseased plants by the total number of plants examined

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

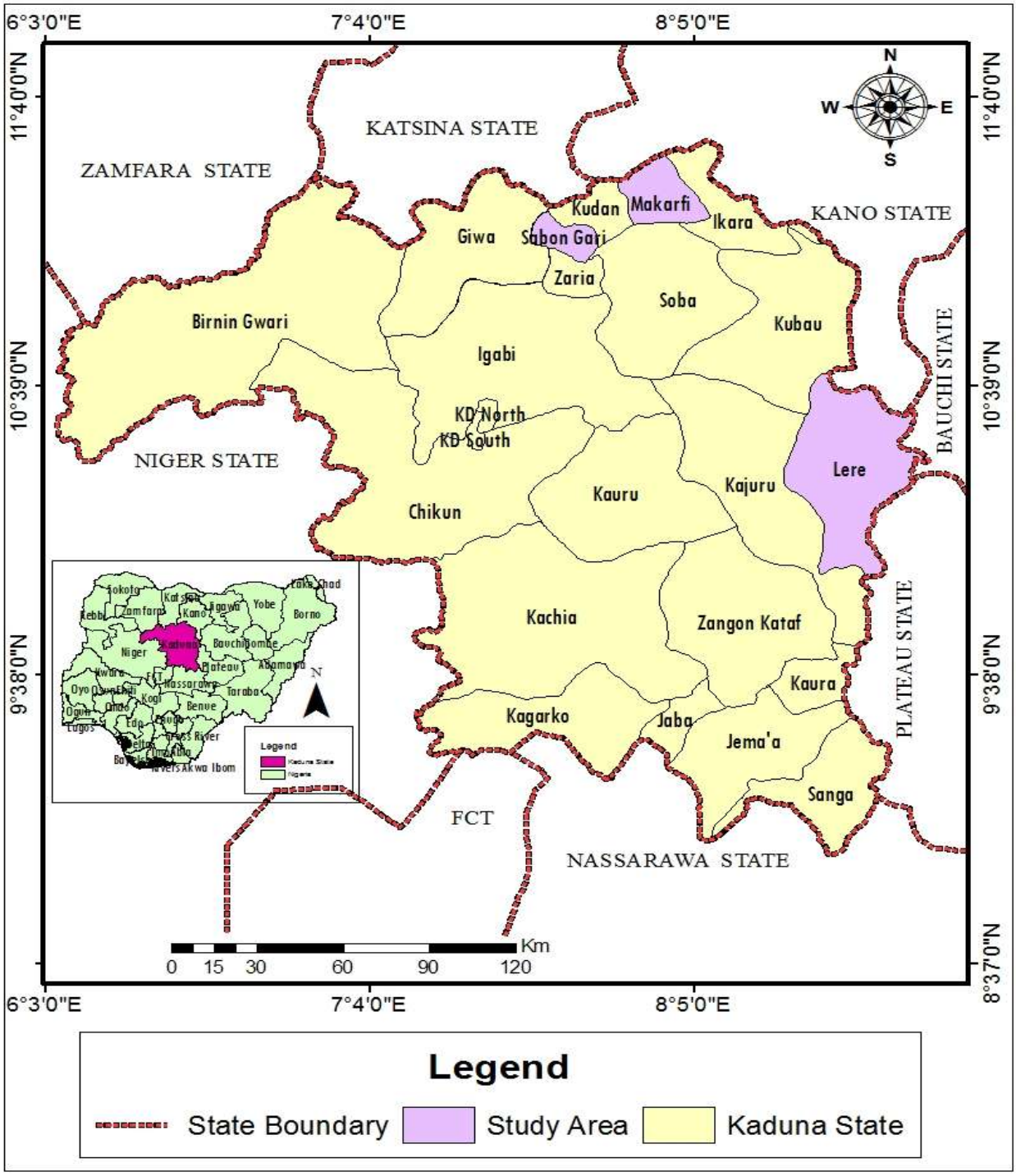


Figure 1: The Local Government Areas surveyed in Kaduna State for maize viruses during the 2018 wet and dry seasons.



Figure 2: The Local Government Areas surveyed in Kano State for maize viruses during the 2018 wet and dry seasons.

The average incidence of each virus disease in each field and LGA was calculated to get the incidence for each State. Other field data collected included: name of LGA and the co-ordinates of the locations which was determined with the aid of Global Positioning System (GPS). A questionnaire (Appendix 1) designed for this study was used to source for information from each farmer on cropping history, cropping pattern, crops surrounding the farm, age of the crop, maize variety, source of seed, symptoms observed on maize plants and weed species were recorded.

3.2 Laboratory Analysis of Samples

3.2.1 Procedure for serological detection of viruses

Maize and weed leaf samples were tested for the presence of *Maize dwarf mosaic virus*, *Sugarcane mosaic virus* and *Maize chlorotic mottle virus.* One gram each of the leaf samples was ground using 1/10 ml of extraction buffer in sterile mortar with pestle to obtain a homogeneous solution.

Serological tests were carried out on the maize and weed samples using the Triple Antibody Sandwich-Enzyme-Linked Immunosorbent Assay (TAS–ELISA) technique for *Maize dwarf mosaic virus* and Double Antibody Sandwich (DAS–ELISA) for *Maize chlorotic mottle virus* and *Sugarcane mosaic virus* as recommended by the manufacturer, DeutscheSammlung von Mikroorganismen und Zellkulturen Braunschweig, Germany.

3.2.1.1Double antibody sandwich enzyme-linked immunosorbent assay procedure was used for the detection of*Maize chlorotic mottle virus* and *Sugarcane mosaic virus*.

The technique was used to test the symptomatic and asymptomatic maize and weed samples for *Maize chlorotic mottle virus and Sugarcane mosaic virus* the antisera were purchased from DeutscheSammlung von Mikroorganismen und Zellkulturen Braunschweig, Germany.

Enzyme-linked immunosorbent assay plates were coated with 200 µl of Polyclonal antibody diluted at 1:1000 in carbonate coating buffer. The plate was covered and incubated at 37^0C for 2 hours, after which plates were washed three times with 0.01 M phosphate-buffered saline (PBS-Tween 20) buffer by flooding for 3 minutes, three times each, draining and tap drying over tissue paper. The dried plate was covered and incubated at 37 ^{0}C for 2-4 hrs.

Samples were extracted in sample extraction buffer by grinding young leaf tips in extraction buffer 1:10 (w/v). 200 µl aliquots of the test samples was added to each well of the antibody coated ELISA plates and then incubated in a refrigerator (4°C) overnight. Then, the plate was washed 3 times with PBS-Tween as described earlier.A measure of 200 µl monoclonal antibody diluted at 1:1000 in conjugate buffer was dispensed in to each well. The plate was covered and incubated at 37 °C for 2 hours. And washed as before.

Poly-nitrophenyl phosphate substrate (200 µl) was added to each well and incubated at room temperature in the dark for 1 hour. The plate was read after 1 hour, using an ELISA reader Readings twice the values of the negative control were considered positive (Clark and Adams, 1977).

3.2.1.2 Triple Antibody sandwich procedure was used for the detection of *Maize dwarf mosaic virus*

Enzyme-linked immunosorbent assay plates were coated with 200µl of polyclonal antibody diluted at 1:1000 in carbonate coating buffer. The plate was covered and incubated at 37 ^{0}C for 2 hours. The plateswere washed 3 times with PBS-Tween by flooding 3 times for 3 mins, drained and tap dried.

A measure of 200µl of 2% skimmed milk was added into each well for blocking of the uncoated sites. The plate was covered and incubated at 37 ^{0}C for 30 min. The blocking solution was then removed and tap dried. Samples were extracted in sample extraction buffer by grinding young leaf tips in extraction buffer 1:10 (w/v). Hundred microliter aliquots of the test samples was added to each well of the antibody coated ELISA plates and incubated in a refrigerator (4 °C) overnight. The plates were washed 3 times with PBS-Tween by flooding 3 times, as described above.

200µl of monoclonal antibody diluted at 1:500 in conjugate buffer was dispensed into each well. The plates were covered and then incubated at 37 ^{0}C for 2 hours after which was washed as described earlier. Goat anti-mouse conjugate labeled with alkaline phosphatase was diluted in conjugate buffer at 1: 1000 (w/v) from which 200µl was dispensed in to each well and incubated at 37 ^{0}C for 2 hours. The content of the plate was emptied and washed thrice with PBS-T.A measure of 200µl of p-nitrophenyl phosphate substrate was added to each well and incubated at room temperature in the dark for 1 hour. The plate was read using an ELISA reader (Clark and Adams, 1977). Optical density values at A_{405nm} twice the value of the corresponding healthy control was considered positive (Kumar, 2009).

CHAPTER FOUR

4.0 RESULTS

4.1 Symptoms of Maize Viruses in the field

The different symptoms observed in the fields included mottling, chlorotic stripes and necrosis.Other symptoms were stunting and abnormal tassels. These symptoms are shown on Plate I. Samples that tested positive for *Sugarcane mosaic virus*, *Maize dwarf mosaic virus*and *Maize chlorotic mottle virus* had one or more of the symptoms mentioned above and the viruses occurred in single and mixed infections.

4.2 Incidence of the Viruses on Maize in Kaduna State

Out of the forty-five samples from Makarfi Local Government Area during the rainy season (Table 1), ELISA showed that twenty-six were positive. *Maize dwarf mosaic virus* and MCMV had the highest incidence of 20% followed by SCMV with 17.8 % incidence. All combinations of the complexes were detected and found to have 2.2% incidence except SCMV+MCMV with 4.4% incidence (Figure 3).

In Sabon Gari LGA, eleven samples were positive, SCMV had the highest incidence of 15.6%, MDMV with 6.7% and MCMV with 2.2% incidence, there were no mixed infections detected (Figure 3).

In Lere Local Government Area, five samples were positive out of the forty-five tested, SCMV had the highest incidence of 6.7% followed by MCMV with 2.2 % incidence. *Maize dwarf mosaic virus*was not detected. SCMV+MCMV was the only mixed infection detected with 2.2% incidence(Table 1, Figure 3).



Plate I: Maize leaves showing symptoms of *Maize dwarf mosaic virus*disease (B) *Sugarcane mosaic virus*disease(C) and *Maize chlorotic mottle virus*diseasae(D) compared with healthy leaf (A)

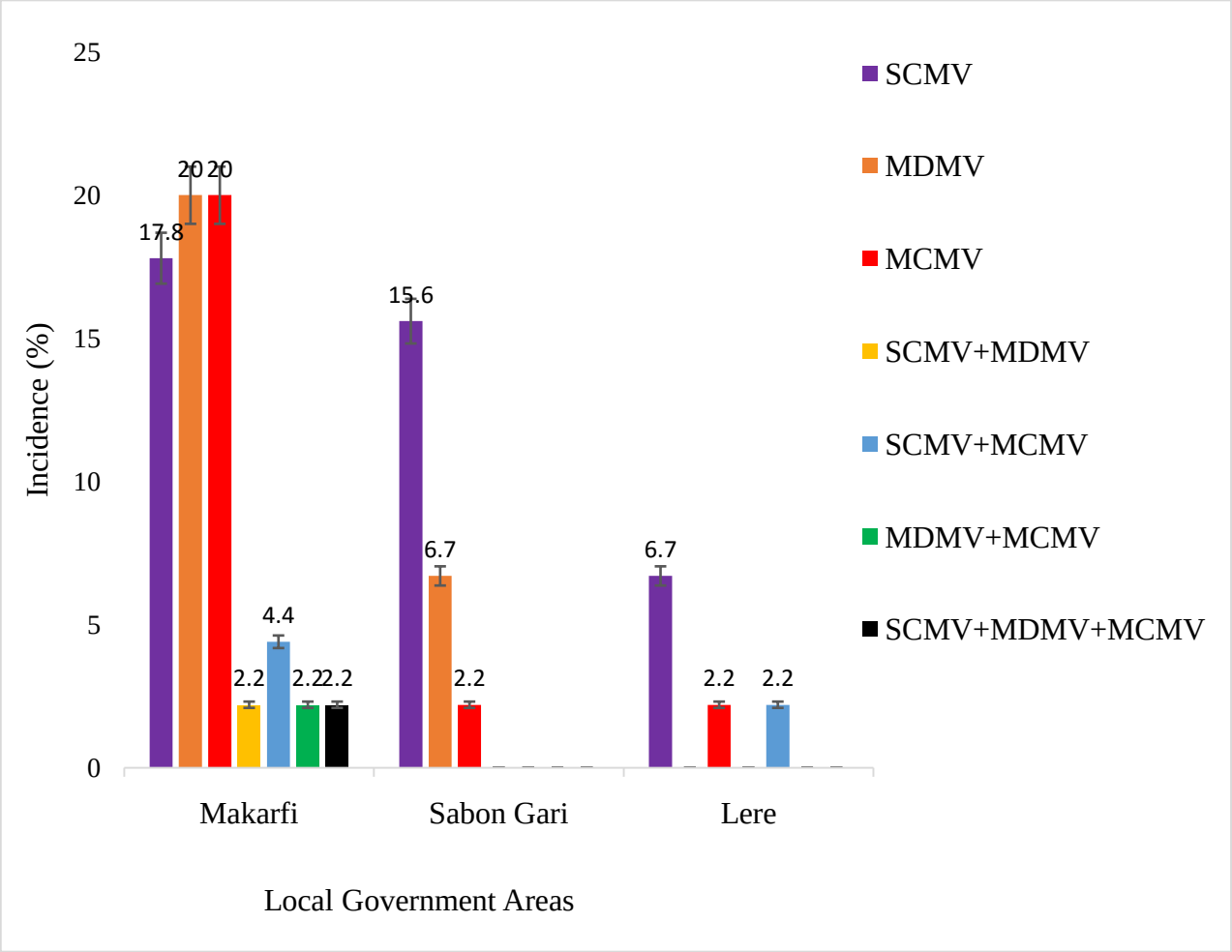


Figure 3: Incidence of the viruses on Maize in Kaduna State during 2018 Rainy Season

In Makarfi Local Government Area,ELISA showed that out of the forty-five dry season samples tested, six were positive. SCMV and MCMV were the only viruses detected with their corresponding complex of SCMV+MCMV and they all had an incidence of 4.4%.

In Sabon Gari Local Government Area, seven samples were positive, SCMV and MCMV had the same incidence of 6.7% incidence but MDMV was not detected, the only mixed infection detected was SCMV+MCMV with 2.2% incidence.

In Lere Local Government Area, eight samples were positive out of the forty-five tested, MCMV had the highest incidence of 15.5% incidence while SCMV had 2.2% incidence. No other single or mixed infections detected (Table 1, Figure 4).

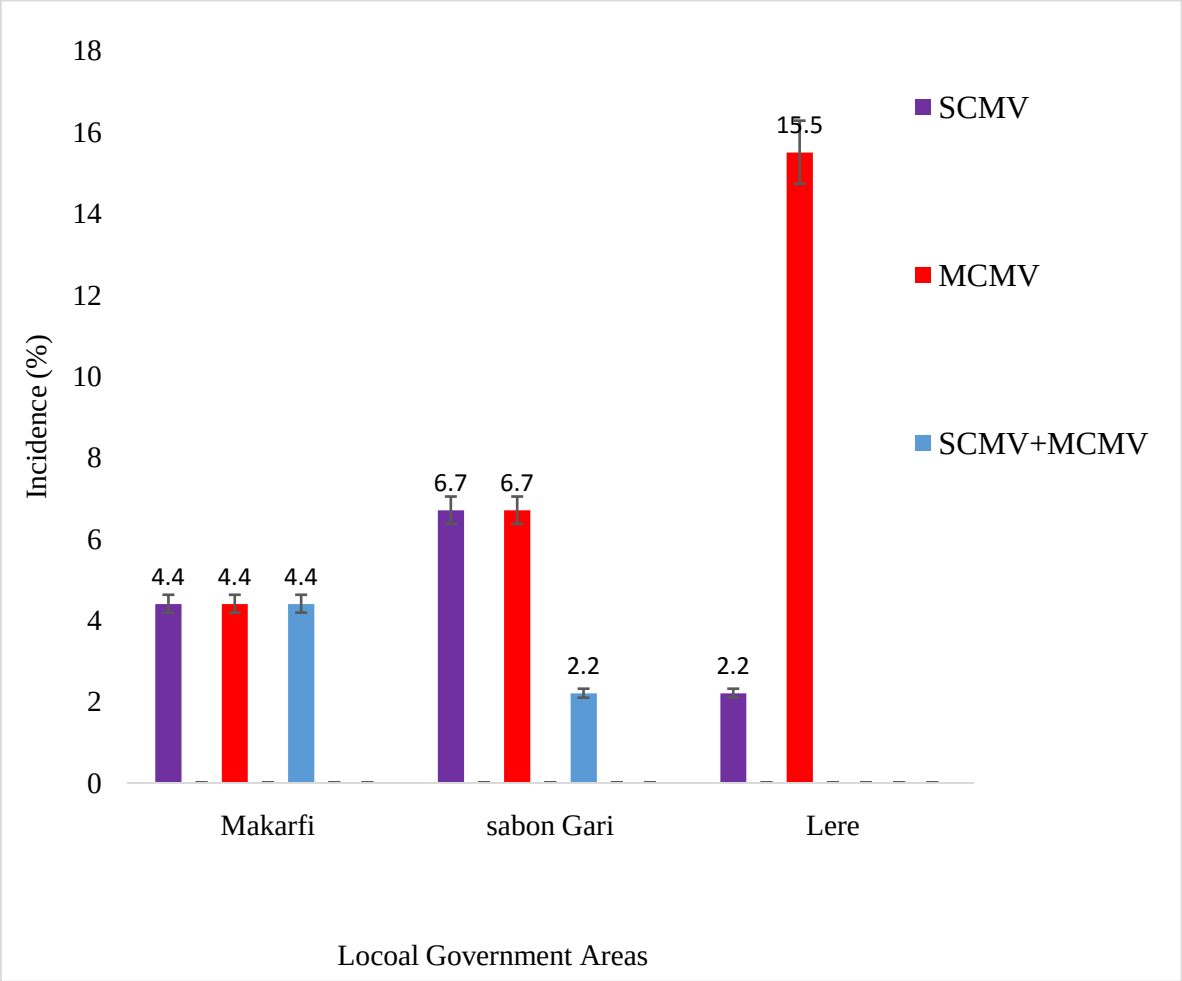


Figure 4: Incidence of the viruses on Maize in Kaduna State during 2018 Dry Season.

4.3 Incidence of the Viruses on Maize in Kano State

Forty-five samples were tested in Kura Local Government Area of Kano State during the wet season, twenty-one samples were positive, and MCMV had the highest incidence of 17.8% followed by SCMV with 13.3%. MDMV had the least incidence of 8.9%. MDMV + MCMV with 2.2% was the only mixed infections detected.

InDambatta LGA, ELISA showed that out of the forty-five samples tested fifteen were positive, SCMV had the highest incidence of 22.2 %,*Maize dwarf mosaic virus* with 4.4 %, *Maize chlorotic mottle virus* was not detected and mixed infection of SCMV+MDMV had 6.7% incidence.

In Bagwai LGA of Kano State sixty samples were tested out of which thirty-two reacted positively with antiserum, SCMV had the highest incidence of 17.8%followed by MDMV with 13.3 % incidence and MCMV with 4.4 % incidence. No complexes were detected (Table 2, Figure 5).

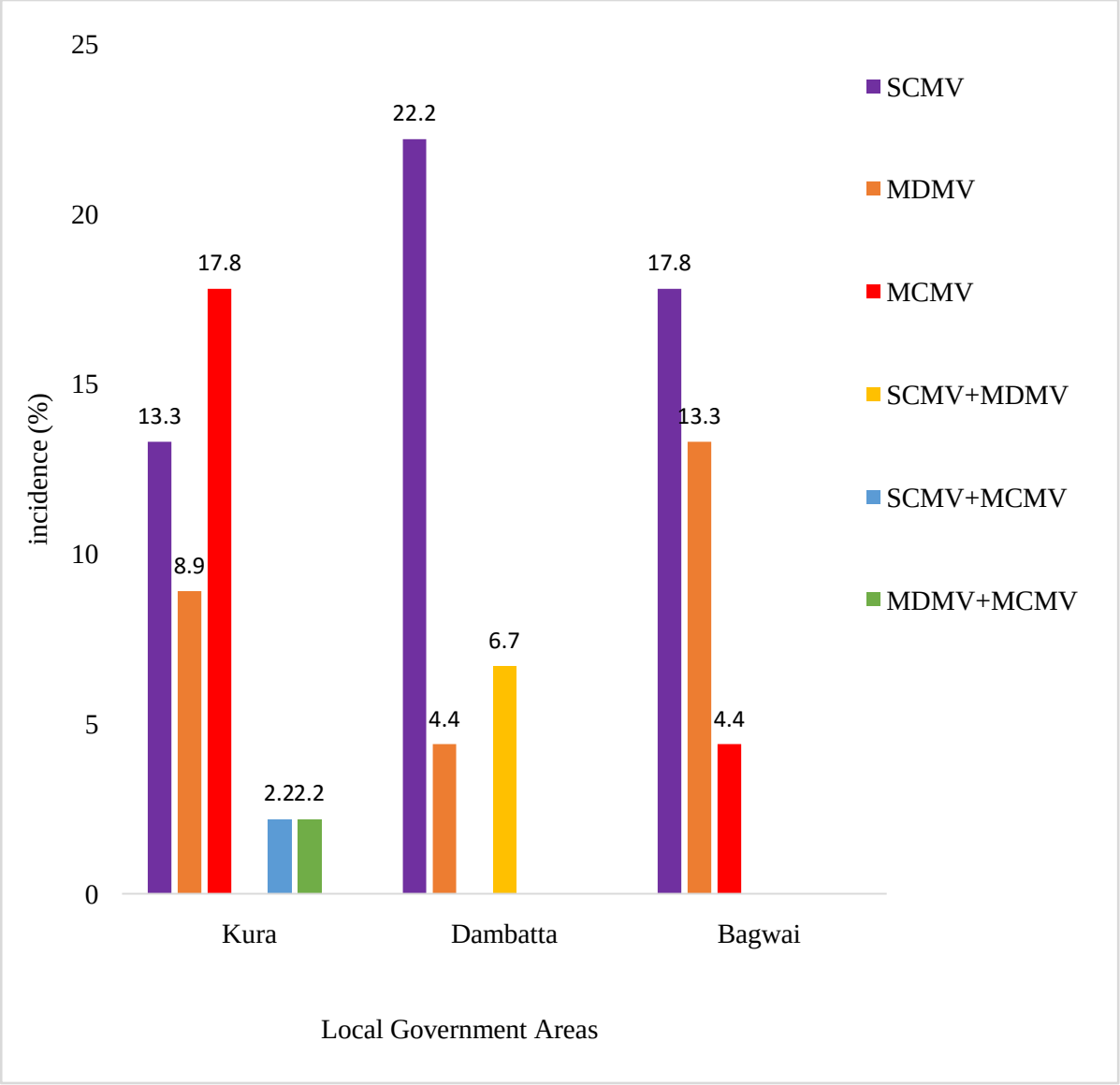


Figure 5: Incidence of the viruses on Maize in Kano State during 2018 rainy season

During the dry season in Kura LGA, forty-five samples were tested, eight were positive, SCMV and MCMV had 8.9% incidence, MDMV was not detected, and the only complex detected was SCMV + MCMV with 4.4% incidence.

Out of the forty-five samples tested from Dambatta Local Government Area during the dry season,four were positive and they were all MCMV with 8.9% incidence, no complexes were detected.

Forty-five samples were tested in Bagwai LGA, thirty-eight samples tested positive. *Sugarcane mosaic virus* had the highest incidence with 37.7%. *Maize dwarf mosaic virus* and MCMV both had 4.4% incidence. The complexes detected were SCMV+MCMV and MDMV+SCMV with same incidence of 2.2% (Figure 5).

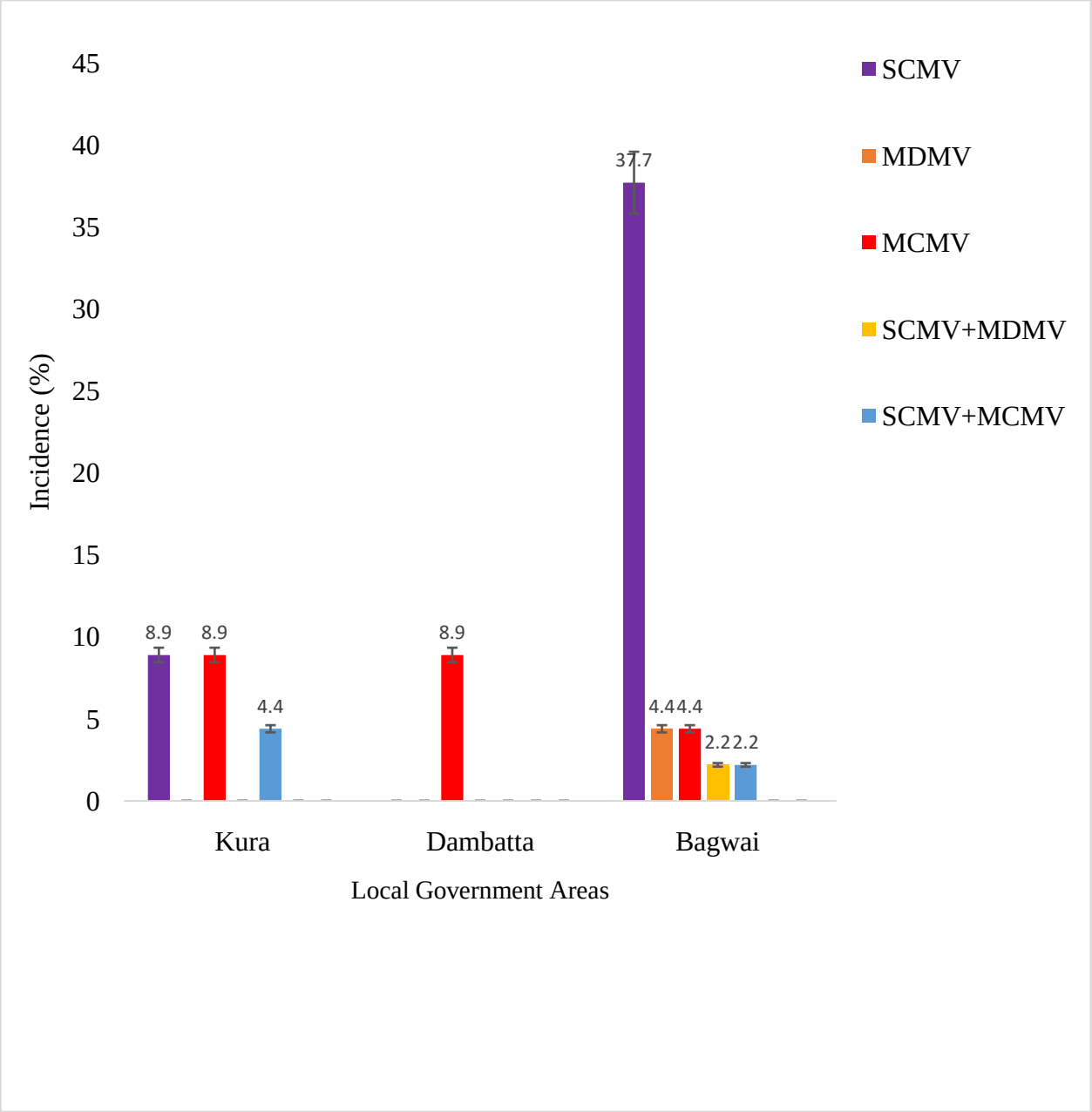


Figure 6: Incidence of the viruses on Maize in Kano State during 2018 Dry Season

4.4 Distribution of the Viruses in Kaduna and Kano States during the 2018 Rainy and Dry Seasons

The distribution of the viruses in Kaduna State varied moderately between farms and considerably between seasons.*Sugarcane mosaic virus* was detected in all six LGAs in both seasons except at Dambatta in Kano State during the dry season,*Maize dwarf mosaic virus* was detected in both States during the rainy season except at Lere where it wasn't detected during the dry season.*Maize dwarf mosaic virus* was not detected in all six LGAs except at Bagwai LGA in Kano State where it was detected, MCMV was present in all six LGAs in both seasons except at Dambatta where it was not detected during the dry season (Tables 1 and 2).

Table 1: Distribution of SCMV, MDMV and MCMV on Maize in KadunaState during the wet and dry seasons

LGA	Location	Number of Maize samples Tested	Number of Positive Samples													
			Single infections						Mixed infections							
			SCMV		MDMV		MCMV		SCMV+MDMV		SCMV+MCMV		MDMV+MCMV		SCMV+MDMV+ MCMV	
			Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry
Makarfi	Angwan mai rijiya	30	3+	-	9+	-	2+	-	1+	-	1+	-	1+	-	1+	-
	Gude Jama	30	3+	-	-	-	3+	-	-	-	-	-	-	-	-	-
	Gazara	30	2+	2+	-	-	4+	2+	-	-	1+	2+	-	-	-	-
Sabon Gari	Mararraban Gwanda	30	3+	3+	1+	-	-	1+	-	-	-	1+	-	-	-	-
	Gwargwaje	30	1+	-	1+	-	1+	-	-	-	-	-	-	-	-	-
	ABU Farm	30	3+	-	1+	-	-	2+	-	-	-	-	-	-	-	-
Lere	Hayin Gada	30	3+	1+	-	-	1+	6+	-	-	1+	-	-	-	-	-
	Abadawa	30	-	-	-	-	-	1+	-	-	-	-	-	-	-	-
	Angwan Makama	30	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Total		270	18	6	12	-	11	12	1	-	3	3	1	-	1	-

SCMV=Sugarcane Mosaic Virus, MDMV= Maize Dwarf Mosaic Virus, MCMV= Maize Chlorotic Mottle Virus, - = Not detected, + = detected

Table 2: Distribution of Sugarcane mosaic virus, Maize dwarf mosaic virus and Maize chlorotic mottle virus on Maize in Kano State during the wet and dry seasons

LGA	Location	Number of Maize Samples Tested	Number of Positive Samples													
			Single Infections						Mixed Infections							
			SCMV		MDMV		MCMV		SCMV+MDMV		MCMV+SCMV		MDMV+MCMV		SCMV+MDMV+MCMV	
			Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry
Kura	Burtalawa	30	3+	3+	1+	-	5+	4+	-	-	-	2+	- -		-	-
	Rinjin Fulani	30	3+	-	2+	-	1+	-	-	-	1+	-	1+	-	-	-
	Dinka	30	-	1+	1+	-	2+	-	-	-	-	-	-	-	-	-
Dambatta	Banloda	30	4+	-	2+	-	-	4+	3+	-	-	-	-	-	-	-
	Hayan Bango	30	6+	-	-	-	-	-	-	-	-	-	-	-	-	-
	Kauyen mai angwar	30	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bagwai																
	Fajin Gini	30	4+	10+	-	-	-	2+	-	-	-	1+	-	-	-	-
	Angwar laraba	30	2+	2+	4+	2+	-	-	-	1+	-	-	-	-	-	-
	Rinji	30	2+	5+	2+	-	2+	-	-	-	-	-	-	-	-	-
Total		270	24	21	12	2	10	10	3	1	1	3	2	-	-	-

SCMV= Sugarcane Mosaic Virus, MDMV= Maize Dwarf Mosaic Virus, MCMV= Maize Chlorotic Mottle Virus, - = Not detected, + = Detected

4.5 Incidence of the Viruses on Weed Hosts in Kaduna State

Fifteen weed samples were tested per Local Government Area (five per farm) during the rainy season. In Makarfi LGA one was positive for MDMV with 6.7 % incidence, in Sabon Gari LGA nine samples were positive for SCMV and MDMV with incidences of 40% and 20%, respectively. In Lere LGA two samples were positive for SCMV, with an incidence of 13.3% (Figure 7).

Out of the fifteen samples tested in each State during the dry season,ELISA results indicated that Makarfi LGA had nine positive samples with SCMV and MCMV having the same incidence of 20%, SCMV+MCMV complex was detected with 6.7% incidence. In Sabon Gari LGA three samples were positive with same single and dual infection were detected as Makarfi with SCMV, MCMV and SCMV+MCMV having an incidence of 13.3%, 6.7%, and 13.3%, respectively. No virus was detected in Lere LGA (Figure 8).

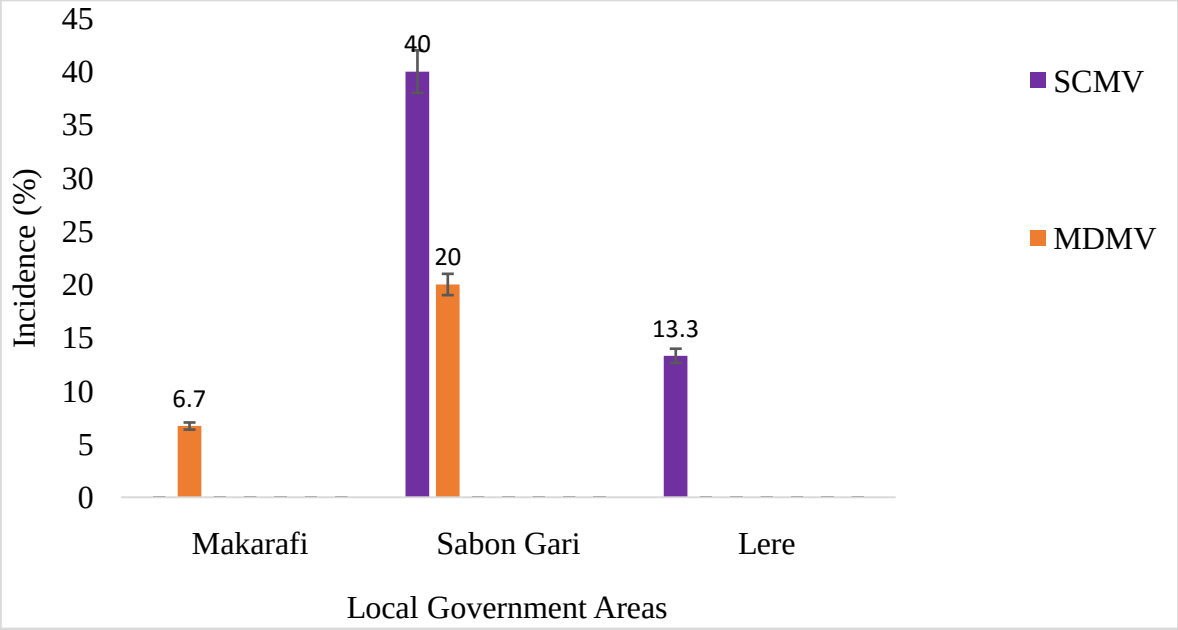


Figure 7: Incidence of the viruses on weed hosts in Kaduna State during the rainy season

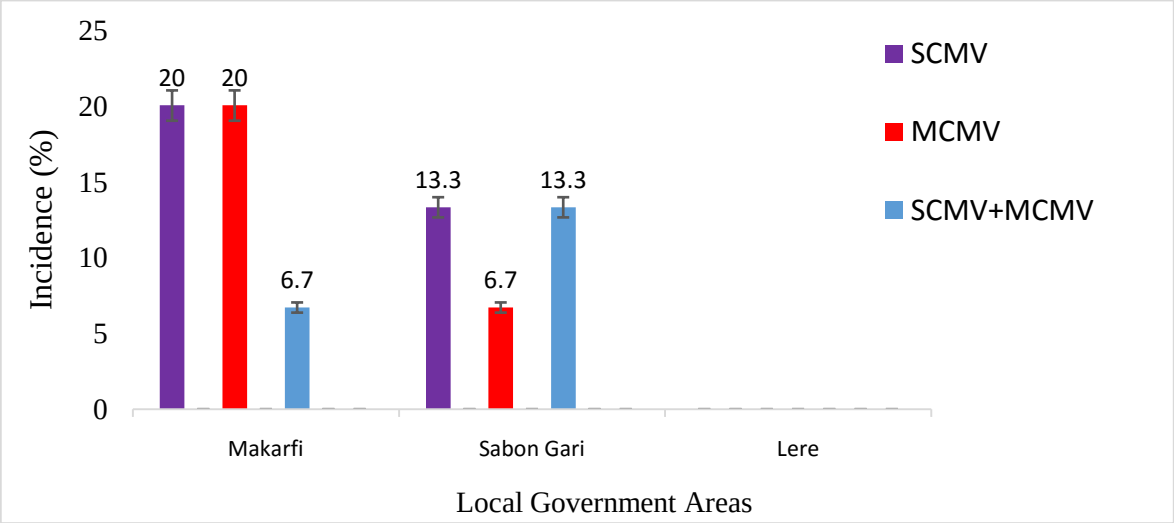


Figure 8: Incidence of the viruses on weed hosts in Kaduna during the 2018 dry season

4.6 Incidence of the Viruses on Weed Hosts in Kano State

In Kura LGA, ELISA results showed that out of the fifteen samples tested in each LGAs, SCMV was not detected, one sample was positive for MDMV with 6.7% incidence, Kura had the highest incidence of MCMV of 40%. In Dambatta LGA only one single infection of SCMV with 26.7% incidence was detected. In Bagwai LGA all three viruses were detected only as single infection, Bagwai had the highest incidence of MDMV with 40% incidence followed by SCMV and MDMV both had 26.7% incidence. No complexes were detected.

Out of the fifteen samples tested in each LGA, ELISA result shows that four were positive in Kura with SCMV and MCMV having 13.3% incidence. No MDMV was detected, while a complex of SCMV+MCMV was detected with 6.7% incidence.

In Dambatta LGA two out of fifteen samples were positive and a single virus (SCMV) detected with 13.3% incidence.In Bagwai LGA fifteen samples were positive out of fifteen tested and SCMV had the highest incidence of 60% followed by MDMV with 26.7% and MCMV with 13.2% incidence. All combinations of mix infections were detected and with same incidence of 6.7%.

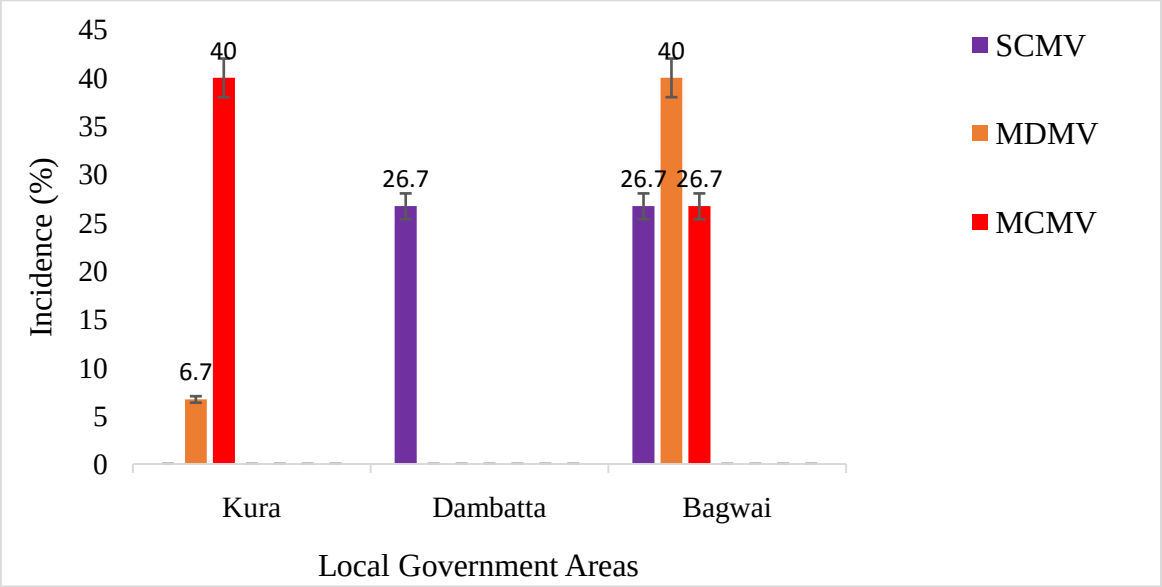


Figure 9: Incidence of the viruses on the weed hosts in Kano during the wet season.

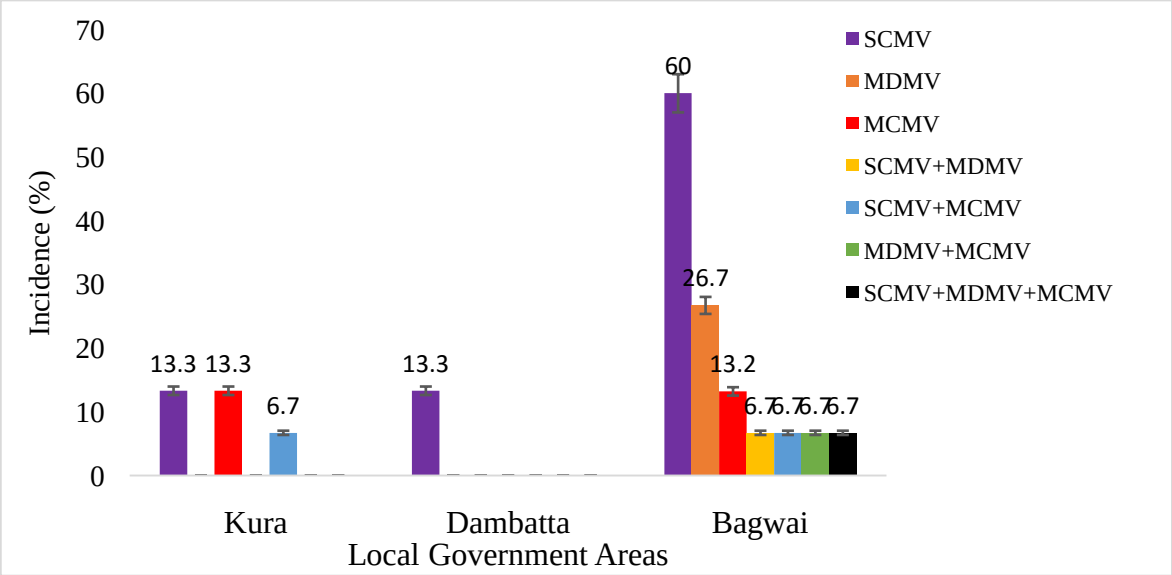


Figure 10: Incidence of the viruses on weed hosts in Kano during the dry season

4.7 Distribution of the Viruses on Weed Hosts in Kaduna and Kano States during the 2018 Rainy and Dry Seasons

SCMV was detected in all LGAs except in Makarfi during wet season and Lere during dry season in Kaduna State, in Kano State SCMV was detected in both seasons and in all LGAs except Kura LGA in Kano State. MDMV was only detected in two LGAs (Makarfi and Sabon Gari LGA) during the rainy season in Kaduna State, in Kano State MDMV was detected in Kura and Bagwai during wet season and was only detected in Bagwai during dry season. Mixed infections of SCMV+MCMV was detected only in the dry season in Makarfi and Sabon Gari in Kaduna State and Kura LGA in Kano State, all other combinations of mixed infections between the viruses studied were detected in Bagwai LGA in Kano State during the dry season (Tables 3 and 4).

4.8Weed Hosts of MLND Component Viruses in Kaduna and Kano States during Rainy and Dry Seasons

During the wet and dry season surveys, a total of 180 samples of other cereals and weeds from within and around the farm were collected and tested for SCMV, MDMV and MCMV.

In Kaduna State, twenty-one weed species out of the ninety samples tested in wet and dry season were found to be virus infected, while in Kano State seventeen out of ninety samples were also detected to be virus infected in both dry and wet season.

ELISA results showed that all the viruses share similar weed hosts from five different families (*Poaceae, Cypereceae, Asteraceae and Rubiaceae*), except SCMV that was detected in (*Gomphrena celosioides*) from the family *Amaranthaceae*.

Table 3: Distribution of Sugarcane mosaic virus, Maize dwarf mosaic virus and Maize chlorotic mottle virus on weed hostsin Kaduna during the 2018 wet and dry seasons

LGA	Location	Number of weed samples Tested	Number of Positive Samples													
			Single infections						Mixed infections							
			SCMV		MDMV		MCMV		SCMV+MDMV		SCMV+MCMV		MDMV+MCMV		SCMV+MDMV+ MCMV	
			Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry
Makarfi	Angwan mai rijiya	10	-	-	-	-	-	1+	-	-	-	-	-	-	-	-
	Gude Jama	10	-	1+	1+	-	-	1+	-	-	-	-	-	-	-	-
	Gazara	10	-	2+	-	-	-	1+	-	-	-	1+	-	-	-	-
Sabon Gari	Mararraban Gwanda	10	-	2+	-	-	-	1+	-	-	-	2+	-	-	-	-
	Gwargwaje	10	5+	-	1+	-	-	-	-	-	-	-	-	-	-	-
	ABU Farm	10	1+	-	2+	-	-	-	-	-	-	-	-	-	-	-
Lere	Hayin Gada	10	1+	-	-	-	-	-	-	-	-	-	-	-	-	-
	Abadawa	10	1+	-	-	-	-	-	-	-	-	-	-	-	-	-
	Angwan Makama	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Total		90	8	5	4	-	-	4	-	-	-	3	-	-	-	-

SCMV=Sugarcane Mosaic Virus, MDMV= Maize Dwarf Mosaic Virus, MCMV= Maize Chlorotic Mottle Virus, - = Not detected, + = detected

Table 4: Distribution of Sugarcane mosaic virus, Maize dwarf mosaic virus and Maize chlorotic mottle viruson weed hosts in Kano State during the 2018 wet and dry seasons

LGA	Location	Number of weed samples Tested	Number of Positive Samples													
			Single infections						Mixed infections							
			SCMV		MDMV		MCMV		SCMV+MDMV		SCMV+MCMV		MDMV+MCMV		SCMV+MDMV+ MCMV	
			Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry
Kura	Burtalawa	10	-	1+	-	-	-	1+	-	-	-	1+	-	-	-	-
	Rinjin Fulani	10	-	1+	-	-	3+	-	-	-	-	-	-	-	-	-
	Dinka	10	-	-	1+	-	3+	1+	-	-	-	-	-	-	-	-
Dambatta	Banloda	10	2+	2+	-	-	-	-	-	-	-	-	-	-	-	-
	Hayan Bango	10	2+	-	-	-	-	-	-	-	-	-	-	-	-	-
	Kauyen Mai Unguwar	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bagwai	Fajin Gini	10	4+	4+	-	2+	-	2+	-	-	-	1+	-	1+	-	1+
	Unguwar Laraba	10	-	3+	3+	2+	2+	-	-	1+	-	-	-	-	-	-
	Rinji	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Total		90	8	11	4	4	8	4	-	1	-	2	-	1	-	1

SCMV=Sugarcane Mosaic Virus, MDMV= Maize Dwarf Mosaic Virus, MCMV= Maize Chlorotic Mottle Virus, - = Not detected, + = detected

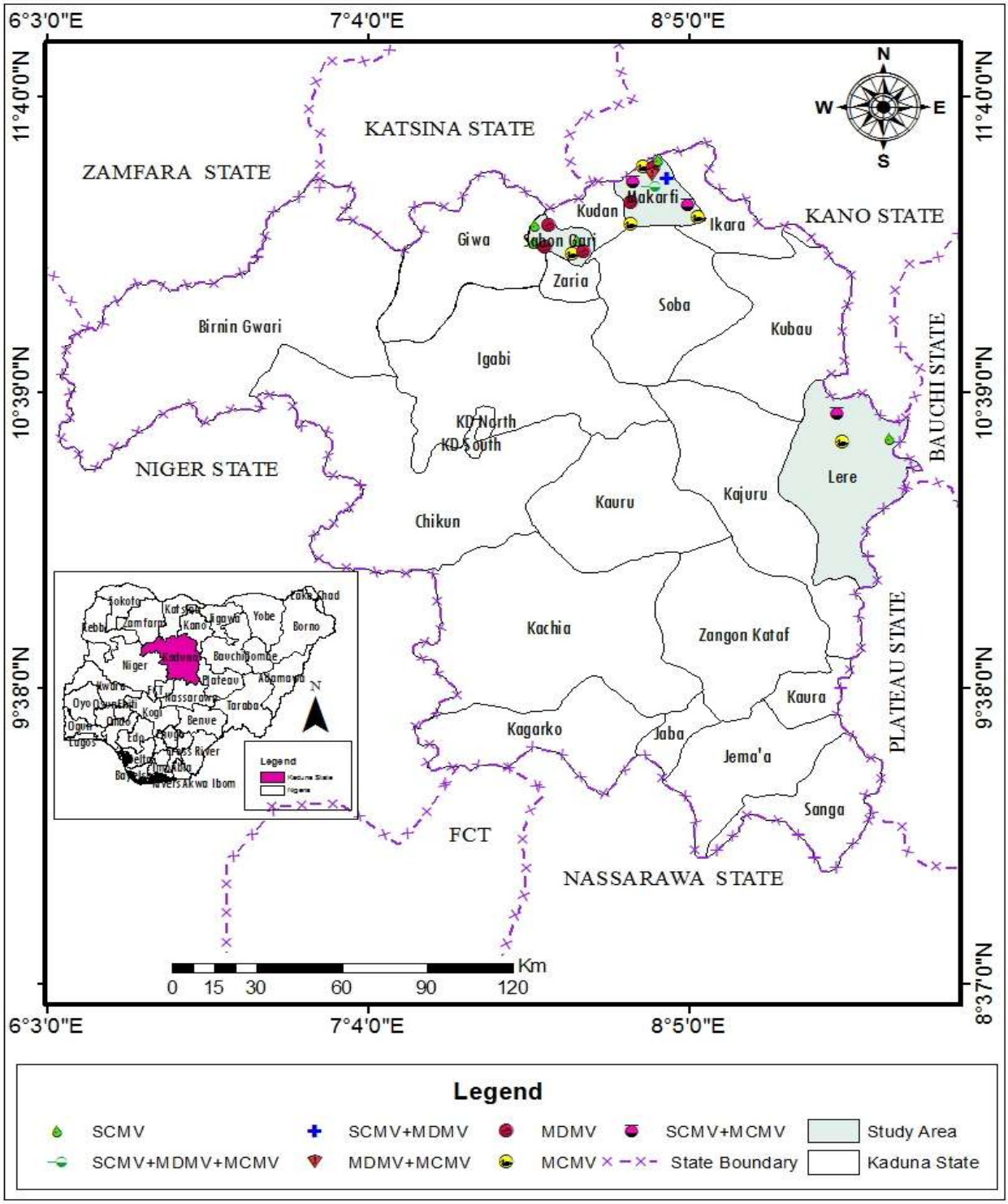


Figure 11: Map of Kaduna State showing the distribution of *Sugarcane mosaic virus*, *Maize dwarf mosaic virus*, *Maize chlorotic mottle virus* and their complexes during the wet and dry seasons

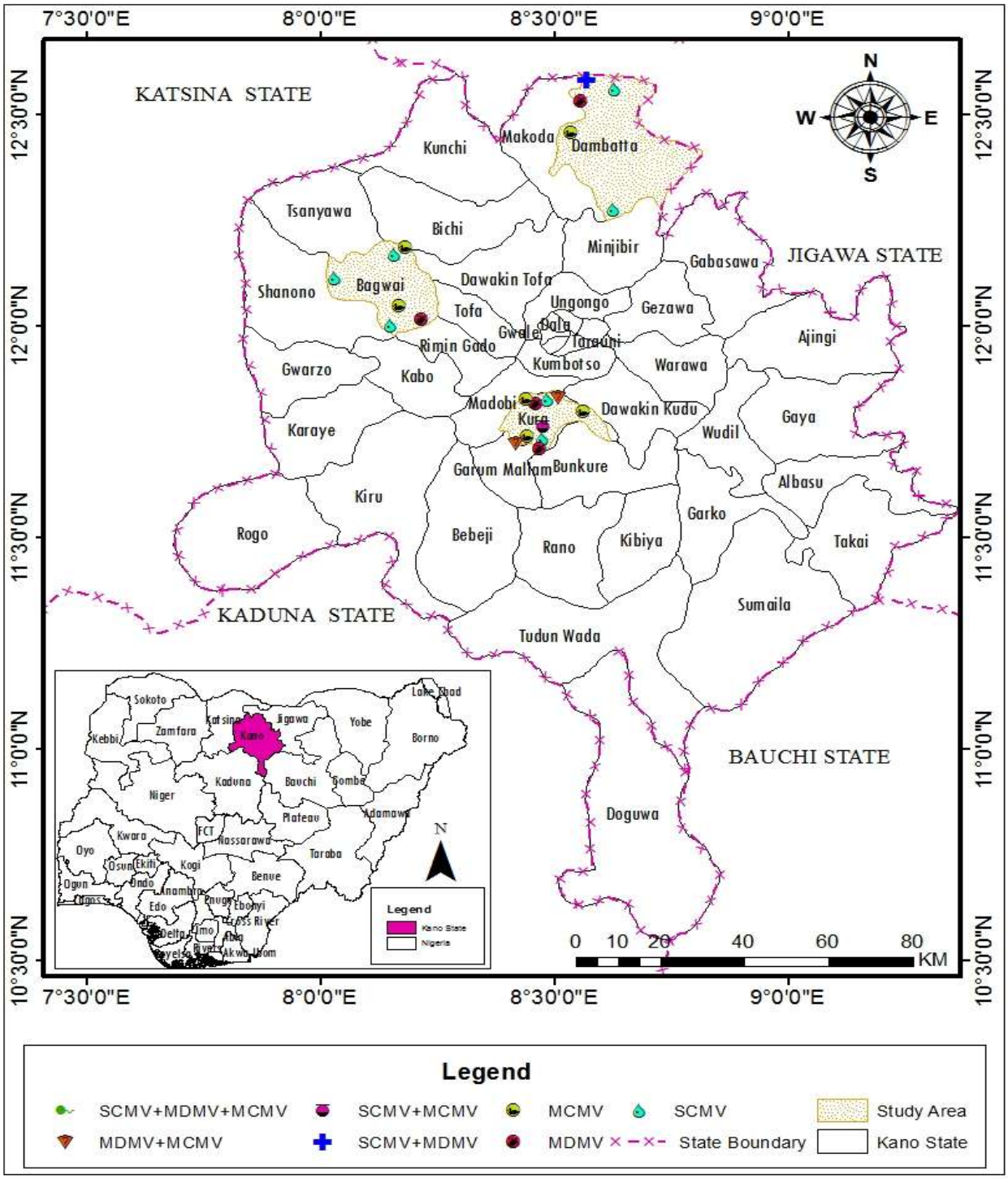


Figure 12: Map of Kano State showing the distribution of *Sugarcane mosaic virus*, *Maize dwarf mosaic virus*, *Maize chlorotic mottle virus* and theircomplexes during the wet and dry seasons

Table 5: Weedhosts of the viruses in Kaduna State

Weed species	SCMV		MDMV		MCMV	
	Wet	Dry	Wet	Dry	Wet	Dry
Poaceae						
ELeusine indica(L.) Gartner.	-	+	-	-	-	+
*Paspalum orbiculare*G.Forst	-	+	-	-	-	-
*Setaria longiseta*P.Beauv.	-	-	+	+	+	-
Cynodon dactylon(L.) Pers.	-	+	+	-	-	-
Chloris pilosa Schumach.	-	-	-	-	-	-
*Saccharum officinarum*Linn.	+	-	-	+	-	-
Dactyloctenium aegyptium (L) Beauv	-	+	-	-	+	-
Setaria verticillata (L.) P. Beauv.	-	+	-	-	-	-
Sorghum bicolor Linn.	+	-	-	-	+	+
Cypereceae						
*Kyllinga squamulata*Thonn. ExVahl	+	+	-	-	+	+
Cyperus rotundus Linn.	+	+	-	-	+	+
Asteraceae						
Vernonia cineria (L.) Less.	+	-	-	-	-	+
*Tridax precumbens*Linn.	-	+	-	-	-	-
Blumea aurita(Lin.f.)	-	+	-	-	-	-
Rubiaceae						
*Spermacoce verticillata*Linn.	+	+	+	-	+	+
Oldenlandia herbacea(L.) Roxb	-	+	-	-	+	-
Amaranthaceae						
Gomphrena celosiodes Mart.	-	+	-	-	-	-
Fabaceae						
Senna obtusifolia Linn.	-	-	+	-	-	-

SCMV= Sugarcane Mosaic Virus, MDMV= Maize Dwarf Mosaic Virus, MCMV= Maize Chlorotic Mottle Virus, - = Not detected, + = Detected.

Table 6: Weedhosts of the viruses in Kano States

Weed species	SCMV		MDMV		MCMV	
	Wet	Dry	Wet	Dry	Wet	Dry
Poaceae						
ELeusine indica(L.) Gartner.	-	+	-	-	-	-
*Paspalum orbiculare*G.Forst	-	+	-	-	-	-
*Setaria longiseta*P.Beauv.	-	-	-	-	-	-
Cynodon dactylon(L.) Pers.	+	-	-	-	-	-
Chloris pilosa Schumach.	-	-	-	-	-	-
*Saccharum officinarum*Linn.	-	-	-	-	-	-
Dactyloctenium aegyptium (L) Beauv	-	-	-	-	-	-
Setaria verticillata(L.) P. Beauv.	-	+	-	-	-	-
*Sorghum bicolor*Linn.	+	-	-	-	-	-
Cypereceae						
*Kyllinga squamulata*Thonn. ExVahl	+	-	-	-	-	-
Cyperus rotundus Linn.	+	-	-	-	-	-
Asteraceae						
Vernonia cineria (L.) Less.	-	-	-	-	-	-
*Tridax precumbens*Linn.	-	-	-	-	-	-
Blumea aurita(Lin.f.)	-	-	-	-	-	-
Rubiaceae						
*Spermacoce verticillata*Linn.	+	+	+	-	+	+
Oldenlandia herbacea(L.) Roxb	-	-	-	-	-	-
Amaranthaceae						
Gomphrena celosiodes Mart.	-	-	-	-	-	-
Fabaceae						
Senna obtusifolia Linn.	-	-	-	-	-	-

SCMV= Sugarcane Mosaic Virus, MDMV= Maize Dwarf Mosaic Virus, MCMV= Maize Chlorotic Mottle Virus, - = Not detected, + = Detected.

CHAPTER FIVE

5.0 Discussion

The objectives of this study were to establish the incidence and distribution of MLND component viruses (SCMV, MDMV, and MCMV) and their Weed hosts in the study area. Maize and weed species were found to harbour SCMV, MDMV and MCMV either singly or as a complex. The symptoms observed on infected plants which included chlorotic leaf mottling and necrotic streaks have been associated with MLND and its component viruses in Hawaii (Nelson *et al.,* 2011), Kenya (Wangai *et al*., 2012), Rwanda (Adams *et al*., 2014) and Congo (Lukanda *et al*., 2014)

The serological tests conducted confirmed that the causal agents of these symptoms were the viruses detected. However, some of the samples with virus-like symptoms tested negative for all viruses studied. These symptoms could have been caused by other viruses whose antisera were not used in this study. Incidence of the viruses varied betweenStates, among Local Government Areas (LGA) and farms in the study locations.

Sugarcane mosaic virus was detected in all six LGAs and in both seasons except, in Danbatta LGA. The incidence was highest in Bagwai LGA of Kano State during the dry season. This could have been due to poor weed management as Bagwai had the most weed infested farms during the survey. In Africa SCMV was first reported by Louie(1980).*Sugarcane mosaic virus*has been reported in Kaduna by Dangora *et al*.(2014). MDMV was detected in all LGAs in Kaduna State during the wet season except in Lere LGA where it was not detected in both seasons. This could be because of low virus concentration as the maize plants from which samples were taken were physiologically matured at the time of visitation.

Tu and Ford(1971) reported that transmission of MDMV was negatively related to leaf age and concentration of the pathogen *in vivo* as the MDMV concentration was lower in older leaves, resulting in decreased aphid transmission. *Maize dwarf mosaic virus* is one of the most widespread Potyviruses infecting maize and sorghum in developing countries (Toler 1985; Loebenstein and Thottappilly 2003). *Maize dwarf mosaic virus*was not detected in all three LGA in Kaduna State during the dry season and its incidence in Kano was also very low during the dry season.This might be because the survey was done in April which is the warmest month in Kano and Kaduna State with an average temperature of 30.9 ^{0}C and 28.6 ^{0}C respectively, Gao *et al*. (2013) reported that The optimal temperature range for aphid growth was 21–27 °C while 30°C was beyond its upper limit for reproduction.Wamaitha *et al*.(2018) reported that both temperature and humidity affect the efficiency and quantity of aphid vectors. *Sugarcane mosaic virus* and MDMV had been detected in Kaduna and Kano by Dangora *et al*(2014)and Muhammad *et al*(2016).

Maize chlorotic mottle virus(MCMV) was detected in some samples from the study areas, this confirmed (Isabirye and Rwomushana, 2017)that lower levels of risk are distributed sparsely across Ivory Coast, Ghana, Nigeria, and Guinea.It was detected in five of the six LGAs in Kaduna and Kano States in both dry and wet seasons.This is because maize production in these areas is all-year round with no Maize-free periods. In this cropping pattern, early planted/dry season maize will serve as a reservoir of both viruses and vectors for a newly planted crop as well as a number of wild grass species (Konate and Traore, 1992; Shepherd *et al.*, 2010). *Maize chlorotic mottle virus*was found to be highest during the wet season in Makarfi LGA (20%) followed by Kura LGA (17.8%)This might be as a result of their

cropping pattern. Farmers in Makarfi and Kura LGAs intercrop their maize with Sugarcane and Sorghum respectively, which are good hosts of SCMV, MDMV and MCMV.

The chlorotic streaks and leaf mottling observed in the field (Plate 1) are characteristics symptoms of MLND causing virus, MCMV, known to cause mottling and mosaic symptoms on maize (Brunt et al., 1990).*Maize chlorotic mottle virus*alone has a big potential to establish in warm-arid, semi-arid and sub-humid tropics (Isabirye and Rwomushana, 2017). *Maize chlorotic mottle virus*is considered a primary disease-causing virus behind almost all MLND cases and alone is capable of leading to the disease development(Mahuku *et al*., 2015).Maizelethal necrosis disease complexes (SCMV+MCMV, MDMV+MCMV and SCMV+MDMV+MCMV) were more widely distributed in maize during the wet season than dry season, while those detected in weeds were more widely distributed during dry season. This indicates that weeds act as alternativesources of virus infection and reservoirs of plant viruses (Duffus 1971;Prajapat *et al*., 2014). *Sugarcane mosaic virus* and *Maize chlorotic mottle virus* (SCMV+MCMV), the most virulent MLN synergistic interaction in Africa, where disease progression and symptoms are greater than the additive effects of each virus individually (Redinbaugh, 2018), was found present in all seasons in Kura, Makarfi and Bagwai LGAs and in both Maize and Weed samples.

Low incidence of SCMV+MDMV+MCMV was also detected in Makarfi maize samples (2.2%) during wet season and Bagwai weed samples (6.7%) in the dry season. This is because the incidence of the three viruses was highest in these areas when compared to other LGAs.The interaction (MCMV+SCMV) in weeds was only detected during the dry season. In Makarfi it was detected in *Cyperus rotundus,* in Bagwai, *Eleucine indica* and *Cyperus rotundus* and in Kura, *Spermacoce verticillata*. This confirmed the experimental inoculations

by Nelson *et al*. 2011) where *Eleucine* , *Setaria*, and *Andropogon* were among the Genera susceptible to MCMV, Positive field infection of these genera (except *Andropogon*) was confrmed by Kusia(2014) in Kenya. Field infection in *Setaria verticillata* has also been detected by (Mariki, 2017). *Maize dwarf mosaic virus*was detected in *Saccharum officinarum*and *Sorghum bicolor* in Makarfi, Kura and Bagwai.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 Summary

Field surveys were conducted on maize farms in 2018 wet and dry seasons to serologically detect the incidence and distribution of the viruses (*Sugarcane mosaic virus, Maize dwarf mosaic virus* and *Maize chlorotic mottle virus*) whose synergistic effect is responsible for the Maize Lethal Necrosis Disease (MLND) wreaking havoc in East Africa, and also to find their alternative hosts in weed leaf samples. A total of eighteen farms were surveyed in Kaduna and Kano States, nine in each state, a total of 540 maize leaf samples and 180 weeds leaf samples were collected during wet and dry season from the two states.Thepresence of these viruses was detected using the Double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) and the Triple antibody sandwich enzyme-linked immunosorbent assay (TAS-ELISA).The virus incidence and distribution in Maize and weed hosts were determined in each LGA of the States. These results confirmed the presence of the three viruses (SCMV, MDMV and MCMV) in both single and mixed infection in all six LGAs.

6.2 Conclusion

- These study established the incidence of the viruses in Kaduna and Kano States during the wet and dry seasons with total values as follows: In Kaduna State: SCMV (14%, 6%), MDMV (8.9%, 0%) and MCMV (6%, 8.9%) and in Kano State: SCMV (17.8%, 20%), MDMV (11.7%, 3.3%) and MCMV (11%, 7.8%).
- This is the first report of MCMV (singly and dually) in Maize and weeds in Nigeria, *Maize chlorotic mottle virus* and *Sugarcane mosaic virus* were detected in samples

showing mild symptoms (necrotic streaks) of MLND in Kura, Makarfi and Bagwai LGAs.

- It was found that the weed hosts of SCMV and MCMV in Kaduna State were *Kyllinga squamulata*, *Cyperus rotundus*, and *Spermacoce verticillata*,while in Kano State only*Spermacoce verticillata* harboured the two viruses.
- MDMV was not detected in Kaduna State during the dry season and its incidence in Kano was also very low during the dry season but was detected in both States during the wet season.

6.3 Recommendations

- Further studies on *Maize chlorotic mottle virus* should be conducted in other agro-ecological zones to establish its distribution in areas with different climatic conditions.
- Molecular tools should be employed to determine the occurrence of the viruses and their variants consideringthe limitations of ELISA.
- Farmers should be continuously educated on the economic importance of viruses, the roles of weed hosts and the significance of weed management.
- More research should be conducted on the interaction of viruses within the hosts to understand the complex relationship as it relates to their transportation, concentration and impact on host performance and growth.

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APPENDICES

Appendix I: Questionnaire

1. State: ……..LGA: …….. Village: ……
2. Name of farmer …….....Gender……
3. Level of Education……Age……
4. Variety of maize…….
5. Size of farm……...
6. Cropping pattern ……
7. If mixed, names of other crops……
8. Do you practice crop rotation? If yes, with what crops? .....
9. Types of crops grown around the farm……
10. Source of your seed? (A) Market vendors (B) Seed Company (C) from previous season crop.
11. Weed species……
12. Age of crop ……..

Appendix II: Information collected from farmers via questionnaires for both dry and wet season in Kaduna and Kano States

Local Government	Location	Farm size (ha)	Cropping pattern	Crop surrounding the plant	Age of plants (months)	Maize variety
Makarfi	Angwan mai rijiya	0.5	Mixed	Sugarcane	2	Hybrid
	Gude Jama	1	Sole	Sugarcane	2	Hybrid
	Gazara	1	Mixed	Tomato	3	Local variety
Sabon Gari	Mararraban Gwanda	1.5	Sole	Onions	2	Hybrid
	Gwargwaje	0.8	Sole	Onions	1	Local Variety
	ABU Farms	2	Sole	Maize	1	Local variety
Lere	Hayin Gada	2	Sole	Onions	2	Hybrid
	Abadawa	2	Mixed	Sorghum	2	Local Variety
	Angwan Makama	2	Sole	Tomato	2	LocalVariety
Kura	Burtalawa	1	Sole	Onions	2	Local variety
	Rinjin Fulani	1	Mixed	onions	1	Local variety
	Dinka	1	Mixed	onions	1	Local variety
Danbatta	Banloda	1.5	Mixed	Onions	2	Hybrid
	Hauyan bango	1.5	Sole	Pearl millet	2	Local Variety
	Kauyen mai Angwa	2	Sole	Tomato	3	LocalVariety
Bagwai	Fajin Gini	0.5	Mixed	Onions	3	LocalVariety
	Angwan Laraba	1	Mixed	Sorghum	3	Localvariety
	Rinji	1	Sole	Pearl millet	3	Local variety

Appendix III: Coordinates, Elevation, farm size and name of villages surveyed

State	Local Government	Village	Coordinates	Elevation	Farm size (ha)
Kaduna	Makarfi	Angwan mai rijiya	N11.37799 E007.86929	638	0.5
		Gude Jama	N11.38450 E007.90267	640	1
		Gazara	N11.37827 E007.89980	650	1
	Sabon Gari	Mararraban Gwanda	N10.989113 E7.4647947	644	1.5
		Gwargwaje	N11.05480 E7.675538	670	0.8
		ABU Farms	N11.178162 E7.6123475	647	2
	Lere	Hayin Gada	N10.403949 E8.6878413	752	2
		Abadawa	N10.39f86 E008.69800	766	2
		Angwan Makama	N10.41601 E008.67254	766	2
Kano	Kura	Burtalawa	N11.786350 E8.424139	459	1
		Rinjin Fulani	N11.7867186 E8.4215728	461	1
		Dinka	N11.7847792 E8.42727123	466	1
	Danbatta	Banloda	N12.42647 E008.48855	459	1.5
		Hayan bango	N12.42158 E008.45524	462	1.5
		Kauyen mai Angwa	N12.42308 E008.48036	465	2
	Bagwai	Fajin Gini	N12.14902 E008.13186	508	0.5
		Angwan Laraba	N12.14358 E 00.13178	505	1
		Rinji	N12.13920 E008.13502	503	1

Appendix IV: Buffers used in the TAS and DAS-ELISA procedure

Buffer	Reagents	Dilution of reagents
Coating buffer	1.59 g sodium carbonate (Na_2C0_3) 2.93 g sodium bicarbonate ($NaHC0_3$) 0.20 g sodium azide (NaN_3)	Dissolved in 900 ml distilled water and filled to one litre. Then adjusted to pH 9.6 (refrigerated)
Phosphate buffered saline	8.0 g sodium chloride (NaCl) 0.2 g monobasic potassium phosphate (KH_2P0_4)	Dissolved in 900 ml distilled water and filled to one litre Then adjusted pH 7.4 with HCl
PBST -Tween (PBST)	PBS + 0.5 ml Tween 20 per litre	0.5 ml Tween 20 was added to 99.5 ml PBS Then adjusted to pH 7.4 with HCl
Sample extraction buffer	PBST + 2% PVP (serva PVP- 15 polyvinyl pyrrolidone)	20 ml of PVP was added to 980 ml of PBST Then adjusted to pH 7.4 with HCl
Conjugate buffer	PBST + 2% PVP + 2% egg albumin (sigma A- 5253)	2.0 g of egg albumin (sigma A-5253) was added to 998.0 ml of sample extraction buffer. Stored at room temperature.
Substrate buffer	97.0 ml diethanolamine 0.2 g sodium azide (NaN_3)	600 ml distilled water was dissolved in and adjusted to pH 9.8 with HCl and filled to one litre