OCCURRENCE, DISTRIBUTION AND ALTERNATIVE HOSTS OF *CUCUMBER MOSAIC VIRUS, WATERMELON MOSAIC VIRUS* AND *ZUCCHINI YELLOW MOSAIC VIRUS* ON CUCUMBER IN KADUNA AND KANO STATES

BY

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A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF A MASTER OF SCIENCE DEGREE IN CROP PROTECTION

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DECLARATION

I declare that the work in this dissertation entitled "Occurrence, distribution and alternative hosts of *Cucumber mosaic virus, Watermelon mosaic virus* and *Zucchini yellow mosaic virus* on cucumber in Kaduna and Kano States" 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.

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(Student) Signature Date

CERTIFICATION

This dissertation entitled "OCCURRENCE, DISTRIBUTION AND ALTERNATIVE HOSTS OF *CUCUMBER MOSAIC VIRUS*, *WATERMELON MOSAIC VIRUS* AND *ZUCCHINI YELLOW MOSAIC VIRUS* ON CUCUMBER IN KADUNA AND KANO STATES" by Grace Egbi ONU 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.

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DEDICATION

This Dissertation is dedicated to my parents Professor Isa and Dr. Rose Onu who never stopped cheering me on.

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ABSTRACT

Field surveys were conducted during the 2020 dry (February to April) and rainy (August to October) seasons in three Local Government Areas each of Kaduna and Kano States of Nigeria to determine the occurrence and distribution of *Cucumber mosaic virus* (CMV), *Watermelon mosaic virus* (WMV) and *Zucchini yellow mosaic virus* (ZYMV) on cucumber. A total of thirty-six farms were surveyed in Kaduna and Kano states, eighteen in each State and a total of 540 cucumber leaf samples and 180 weeds leaf samples were collected during dry and rainy season from the two states. All the samples were screened by Double Antibody Sandwich- Enzyme-Linked Immunosorbant Assay (DAS-ELISA). All the three viruses were detected in all the Local Government Areas visited. In Kaduna State *Cucumber mosaic virus* had an incidence of 10.37 % during the dry season and 16.29 % at the rainy season, *Watermelon mosaic virus* had an incidence of 3.67 % in the dry season and 4.44 % during the rainy season, *Zucchini yellow mosaic virus* had an incidence of 0.73 % at the dry season and 5.18 % during the rainy season respectively. In Kano State *Cucumber mosaic virus* had an incidence of 5.18 % at the dry season and 18.53 % at the rainy season, *Watermelon mosaic virus* had incidences of 11.58 % at the dry season and 1.48 % during the rainy season while *Zucchini yellow mosaic virus* had incidences of 0.74 % during the dry season and 4.44 % during the rainy season respectively. Mixed infections were also observed among CMV+WMV with incidences of 4.45 % during the dry season and 2.22 % at the rainy season in Kaduna. CMV+WMV (4.44 %) and CMV+ZYMV (2.33 %) was only observed during the rainy season in Kano State.

This is the first report of ZYMV on cucumber in Nigeria, CMV was the most widespread and prevalent virus infecting cucumber and weed in the study areas. This is also the first reports of

Ageratum houstonianum and *Alternanthera brasiliana* as weed hosts of CMV and WMV in Kaduna and Kano States respectively.

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CHAPTER ONE

1.0 INTRODUCTION

Cucumber (*Cucumis sativus* L.) is a major vegetable crop with important economic and biological value (Che and Zhang, 2019). It is a versatile fruit vegetable consumed in various ways and the only cucurbit crop grown extensively in green-houses (Ni and Punja, 2019). It can be grown year because of its commercial importance and also its high export potential, worldwide (Rahman *et al*., 2016; Zitter and Murphy, 2009). Cucumber together with gherkins is the third global leading vegetable in terms of production with 87.81 million metric tonnes (MMT) produced on 2.2 million hectares globally (Statista, 2019). It is a warm season crop, requiring slightly milder climate than watermelon (Ni & Punja, 2019). It has been successfully cultivated in tropical, sub-tropical and temperate regions of the world but is sensitive to cold weather. It is a soft succulent plant with high water content and has large leaves that form canopy over the fruits. The vines grow on stakes or on trellises and the fruit is roughly elongated with tapered ends, which are used in unripe mature state, usually eaten raw in salads or pickled and are also stewed in tropical regions (Grubben *et al*., 1977).

Cucumber is an economic crop in Nigeria which is not only eaten to overcome nutritional deficiency but is also a good sources of income for farmers (Elum *et al*., 2016). Cucumbers are a rich source of conventional antioxidant and nutrients including vitamin C, beta-carotene, and manganese (Timothy *et al*., 2019). They are well known for their nutritional value. More than 90% content of cucumber is water, besides its low caloric value it is very rich in potassium and folic acid but low in sodium. It also contains carotenoids when consumed with the skin. It does well on well-drained fertile soils with pH 6.0 -7.0 and very rich in organic matter (Eifediyi and Remison, 2010). Cucumber are important crops cultivated around the globe in wide geographic

regions (Wang *et al*., 2018). It is often planted on raised beds and thrives in sandy loam soils (Palukaitis *et al*., 1983). The crop requires a good amount of sunshine and warmth (Jeffrey, 2001). Cucumber requires a warm climate with optimum temperature for growth at about 30^0 C. The optimum night temperature is between 18-21^0 C while the minimum temperature for its development is 15^0 C. At temperature less than 10^0 C, growth stops while at 0^0 C all plants perish (Khurana and Singh, 2001). Cucumber is extensively grown throughout the world especially the slicing types. China is the largest producer of cucumbers producing 51% (70,288,130 MT) of the total world production. The other important cucumber producing countries include Iran, Turkey, the USA, India and Russia (FAOSTAT, 2019).

Production figures in Africa showed that, cucumber was cultivated on 409,547 hectares with a production of 1,433,124 MMT with Egypt being the largest producer (FAOSTAT 2019). In Nigeria, Cucumber is grown mostly in the northern States like Plateau, Kaduna and Kano (NIHORT, 2018).

Cucumber is a rich source of important nutrients and bio-active compounds, it is not only used as food for nourishment but also in therapeutic medicine and beauty culture applications since ancient times (Dixit and Kar, 2010). It is also rich in moisture content and low in calories (Mukherjee *et al*., 2013). It is considered a vegetable crop which is rich in polyphenolics and other phytochemicals (Agatemor *et al*., 2018), that are known to possess multiple biological activities such as antioxidant, anti-carcinogenic, anti-elastase, hypolipidemic, anti-inflammatory, diuretic, amylolytic, antimicrobial, and analgesic activities (Grubben and Denton, 2004).

1.1 JUSTIFICATION

Cucurbits virus diseases is a worldwide problem that causes yield loss as high as 100 % (Al-Ali *et al*., 2013). Viruses badly affect cucumber cultivation on a worldwide basis. The important

viruses of *Cucurbitaceae* family are *Zucchini yellow mosaic virus* (ZYMV), *Watermelon mosaic virus* (WMV), *Papaya ring spot virus* (PRSV), *Cucumber green mottle mosaic virus* (CGMMV), *Cucumber mosaic virus* (CMV), *Melon necrotic spot virus* (MNSV). CMV, WMV and ZYMV are among the most important viruses having a wide host range.

Despite the growing relevance of cucumber, low yields are obtained in farmer's farms and its production in Nigeria is constrained by scarcity of healthy planting seed, lack of capital, high fruit perishability, climatic factors and plant pests and diseases (Umeh and Ojiako, 2018). Viruses are major constraints in the cultivation of cucumber worldwide (Alabi *et al*., 2011; Anderson *et al*., 2004; Tzanetakis *et al*., 2013). Virus diseases are a major challenge to cucumber production, with more than 20 viruses reported to infect cucumber worldwide (Lecoq and Katis, 2014). They have an important limitation in production of cucumber and can cause massive damage to the crops and prevent the growth of cucumber crops in certain areas.

Virus diseases are a worldwide problem of cucurbits and a major limiting factor for cucumber production (Ayo-John *et al*., 2014). Virus diseases among other biotic and abiotic factors present consequential challenge to the successful production of cucumber. Some of these viruses have a worldwide distribution such as *Cucumber mosaic virus* (CMV), *Watermelon mosaic virus* (WMV) and *Zucchini yellow mosaic virus* (ZYMV) (Chikh-Ali *et al*., 2019). A number of biotic (weed, insects, fungi, bacteria etc.) and abiotic (sunlight, temperature, rainfall, humidity etc.) diseases are important limiting factor that badly affect the yield of cucumber. The reason for low yields of cucumber is the lack of awareness about some of (WMV and ZYMV) the diseases and agronomic practices that provides a favourable environment for rapid development of the disease. Cultural practices adapted by the farmers, lack of awareness of farmers about viral diseases, their control, and high population of weeds are some of the major contributing factors

resulting in high incidence of the virus diseases (Ayo-John *et al*., 2014). Yield loss of 10-20 % are common and losses of up to 100 % can occur with *Cucumber mosaic virus* (Ayo-John *et al*., 2014). Seminis (2018) has reported yield loss of 50 % on cucumber caused by *Watermelon mosaic virus* while early infection before flowering by *Zucchini yellow mosaic virus* causes yield loss of up to 100 %, Ayo-John *et al*s (2014) reported CMV (100 %), WMV (100 %) incidence on cucumber in South-west Nigeria.

Infected weeds could serve as initial or primary sources of inoculum to the healthy cucumber plants. They can also serve as alternative hosts of the viruses and or insect vectors within crop fields and neighboring crops, thereby making the management of viruses and their vectors difficult (Alegbejo and Banwo, 2005; Asala *et al*., 2014).

Kaduna and Kano States are among the top Cucumber producing states in North-western Nigeria (NAERLS, 2019). However, little or no information exists on the incidence, distribution and weed hosts of CMV, WMV and ZYMV in any of these states. There is therefore the need to determine the incidence, distribution and weed host of these viruses in Kaduna and Kano States.

1.2 Objectives of study

The objectives of this research are to:

i. Determine the incidence and distribution of *Cucumber mosaic virus* (CMV), *Watermelon mosaic virus* (WMV) and *Zucchini Yellow Mosaic Virus* (ZYMV) in cucumber in Kaduna and Kano States.
ii. Identify the weed hosts for *Cucumber mosaic virus*, *Watermelon mosaic virus* and *Zucchini Yellow Mosaic Virus* in these Kaduna and Kano States.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Origin and Distribution of Cucumber

Cucumber (*Cucumis sativus* L.) is an important vegetable and one of the most popular members of the Cucurbitaceae family (Lower, 1986). It is believed to have originated in India where it has been grown for thousands of years (Zeven and De-Wet, 1982). *Cucumis sativus* var. hardwickii, a wild taxon native to India, has been proposed as the wild progenitor of the domesticated forms of C. *sativus*. It spread to China and Greece from India about 2,000 years ago (Robinson and Decker-Walters, 1997; Whitaker and Davis, 1962). Cucumbers, like cantaloupes, squash, pumpkins and watermelons, are members of the cucurbit family. It is a creeping vine that roots in the ground and grows up trellises or other supporting frames, wrapping around supports with thin, spiralling tendrils (Elum *et al*., 2016).

2.2 Botany and Morphology of Cucumber

Cucumber belongs to the *Cucurbitaceae* (gourd family) and genus *Cucumis* of which there are 20 to 25 species found mostly in Asia and Africa (Robinson and Whitaker, 1974). The root systems are extensive although shallow. Stems are square with stiff bristle hairs, unbranched tendrils and generally range in length from 0.4 to 3 m. Tendrils at each node help anchor plants and allow climbing on supports. Cucumber petioles are 3–15 cm long, having a rough leaf blades with triangular ovate shape from 5 to 25 cm wide having three- to five-angled regions or shallow lobed sinuses and a pointed apex (Rubatzky and Yamaguchi, 1997).

2.3 Climatic Requirement for Cucumber Production

Cucumber requires a warm climate. In cold countries it can be grown only in greenhouses or in open field if there are hot summer days. The optimum day temperature is 30^0C, and optimum night temperature is 18-21^0C. It requires a minimum temperature of 15^0C for its efficient development (Chinatu *et al*., 2016).

2.4 Nutritional Composition of Cucumber

Cucumber which are grown for eating are called slicers and those intended for pickling are called picklers (Grubben *et al*., 1977). Although it is less nutritious than most fruits, it is still a very good source of phytonutrients such as flavonoid, betacarotene, triterpene, lycopene, lignin vitamins A, C, K, B6, potassium and also provides dietary fibres, pantothemic acid, magnesium, phosphorus, copper and manganese(Vimala *et al*., 1999). It contains ascorbic acid and caffeic acid both of which helps to smoothen skin irritation and reduces swelling in skin. Its juice is often recommended as a source of silicon to improve the complexion and health of the skin (Duke, 1997).

The nutritional composition of a 100g portion of cucumber is composed of most of its weight in water with proteins, fat and carbohydrates as primary metabolites and also dietary fiber which plays an important role in the digestive system (Sotiroudis *et al*., 2010). The nutritional benefits of cucumber in terms of micronutrient contributions are notable, it generally contains water (95%) and minute amounts of protein (0.6%), lipids (0.1%) and carbohydrates (2.2%) (Uthpala *et al*., 2020).

2.5 Cucumber Production

FAOSTAT (2018) reported that the estimated world production for cucumber in 2018 was 72,219,440 metric tonnes. China was by the largest producer accounting for nearly 75% of

global production at 56,240,428 MT, followed by Iran at 2.283.750 MT and third largest producer Turkey at 1,848,272 MT. In 2019 Statista reported world production of Cucumber at 87.81 MMT.

2.6 Constraints to Cucumber Production

Cucumber is susceptible to a number of diseases caused by fungi, bacteria and viruses. Most diseases begin on plants growing in the field, and continue to develop on the fruit during storage and transit. Insect pest and nematode infestations in cucumber bring about heavy losses through yield reduction, lowered quality of produce, and an increased cost of production (Sharma *et al.*, 2016). Weeds compete with cucumber for space, nutrients and water, they also serve as host for viruses in crops. Some viruses infecting cucumber include *cucumber mosaic virus* (CMV), *Watermelon mosaic virus* (WMV), *Zucchini yellow mosaic virus* (ZYMV),

2.6.1 Insect Pests of Cucumber

Insects and mites cause severe problems in the production of cucumber, either through direct damage to the crop or through transmission of disease agents, such as the aphid-borne mosaic viruses. Common insect pests of cucurbits are: Silverleaf Whitefly (*Bemisia tabaci* Gennadius), Pickleworm (*Diaphania nitidalis* Stoll), Melonworm (*Diaphania hyalinata* Linnaeus), Cucumber Beetles (A*calymma vittatum* (Fabricius), *Diabrotica undecimpunctata howardi* Barber), Thrips (Melon Thrips *Thrips palmi* Karny; Tobacco Thrip *Frankliniella fusca* Hinds), Leafminers (*Liriomyza sativae* Blanchard, *L. trifolii),* Striped (*Acalymma vittatum*) and Spotted beetles (*Cerotoma trifurcate* Forster*),* Aphids (primarily *Aphid gossypii*) (Webb and Leng, 1993).

2.6.2 Diseases of cucumber

2.6.2.1 Fungal diseases of cucumber

Fungal diseases are a primary limiting factor in commercial production of cucumber in field and greenhouses. Infection by these pathogens can result in plant death and reduced yields.

Anthracnose

Anthracnose disease of cucurbits caused by *Colletotrichum orbiculare* is most commonly found on cucumber, melon and watermelon. Symptoms on leaves begin as water-soaked spots which typically become yellowish in appearance on cucumber and melon or dark brown to black on watermelon. These spots eventually turn brown and may expand over the leaf surface. Foliar lesions are not restricted by leaf veins and often have cracked centres. Infected petioles and stems may develop shallow, elongated, tan lesions on melon but the lesions are less obvious on cucumber. Stem lesions on melon can girdle the stem and cause plant wilting. Infected fruit develop circular, sunken, blackish lesions where tiny fruiting bodies (acervuli) may develop. Under humid conditions, the fruiting bodies produce conidia which give the lesions a pinkish-salmon colour, which is very characteristic of this disease. When pedicels of young fruit become infected, the fruit may shrivel and abort (Seminis, 2015).

Powdery mildew of cucurbits

Powdery mildew affects almost all cucurbits under field and greenhouse conditions. It is caused by airborne fungi *Sphaerotheca fuliginea* [(Schlect. ex. Fr.) Poll.] and *Erysiphe cichoracearum* (D. ex. Merfat). The disease is widely distributed and very destructive on cucumber in most areas of the world. It is a major production constraint causing yield losses of 30 %–50 % (El-Naggar *et al*., 2012).

2.6.2.2 Bacterial diseases of cucumber

Bacterial wilt

Bacterial wilt caused by *Erwinia tracheiphila* Smith and are transmitted by the vectors: *Acalymma vittatum* Fabricius (striped cucumber beetle) and *Diabrotica undecimpunctata howardi* Barber (spotted cucumber beetle) The disease is severe on cucumber causing up to 60% loss in field, but is less damaging to watermelon. Symptoms begin with wilting, which may be confined to individual runners or may involve the entire plant. Plants may wilt at any growth stage, but wilting is often most severe during periods of rapid growth. Affected leaves display marginal chlorosis and necrosis, eventually the entire plant becomes necrotic and dies. (Seminis, 2015).

Angular Leaf Spot

Angular leaf spot caused by *Pseudomonas syringae pv. Lachrymans* is found in all cucurbit growing regions. The disease can occur on most cucurbits but is of greatest importance on cucumbers. Foliar symptoms initially appear as small, water-soaked areas on the underside of the leaf, which develop an angular appearance due to restriction by the small leaf veins. Under humid conditions, a milky exudate may appear from the water-soaked areas on the lower leaf surface. As this exudate dries, a white crust is left behind. Leaf spots turn brown and may develop yellow haloes. The centres of the spots may eventually disintegrate, giving leaves a tattered appearance. Infection on stems, petioles and fruit first appears as water-soaked spots, which may also produce the milky exudate under humid conditions and corresponding white crust upon drying. Infection of young fruit may result in deformation at maturity. Secondary soft rots often develop on infected fruit (Seminis, 2015).

2.6.3.3 Nematodes

Root-Knot Nematode

Cucumber is susceptible to Root-knot caused by *Meloidogyne*. Affected plants appear stunted, foliage takes on a pale green to yellow appearance. Infected plants wilt during the hottest periods of the day due to reduced transpiration. Although the crop may look healthy in appearance throughout the growing season, yield and quality of the fruit may be greatly reduced. In heavy infections, plants completely wilt and dies as the nematode population increase. When infected plants are removed from the soil, knobby, wart-like galls caused by the nematode can be seen singly or in clumps on the roots (Seminis, 2015).

2.6.2.3 Viral diseases of Cucumber

Cucumbers are very sensitive to viral infection and a complex of viruses are able to infect cucurbits (Zitter *et al*., 1996). Majority of these viruses cause huge losses in cucurbit production. The important viruses are *Cucumber mosaic virus* (CMV), *Squash mosaic virus* (SqMV), *Watermelon mosaic virus* (WMV), *Zucchini yellow mosaic virus* (ZYMV), and *Papaya ring spot virus* (PRSV), *Cucumber green mottle mosaic virus* (CGMMV) (Tobias and Tulipan, 2001). According to the different estimates, 3-5 % of overall vegetable production is lost due to virus infections, but losses can be occasionally very high, where pest control is insufficient, especially in developing countries (Yeşil, 2014).

Cucumber Green Mottle Mosaic Virus

Cucumber green mottle mosaic virus (CGMMV), a member of the genus Tobamovirus is an economically important seed transmitted virus infecting many cucurbit species. Crop yield losses of up to 15% have been reported in India (Antignus *et al*., 1990, 2001). CGMMV causes vein clearing and crumpling on young leaves while mature leaves become bleached and chlorotic.

The disease can be very problematic due to the speed at with which it is transmitted and its stability and long viability in plant debris (Ling *et al*., 2014).

Cucumber Mosaic Virus

Cucumber mosaic virus (CMV), a member of *Bromoviridae* family is an important pathogen in agricultural crops which spreads throughout the world. CMV has a wide range of hosts and attacks a great variety of vegetables, ornamentals, and other plants. Its host range includes 1200 species in over 100 plant families (Palukaitis and García-Arenal, 2003; Zitter and Murphy, 2009) and vectored by more than 80 aphid species. The disease causes up to 60% loss in yield in developing countries. It is transmitted primarily by aphids, seeds, cucumber beetles, parasitic plants and mechanically. In agricultural crops, especially cucurbits (*Cucurbitaceae* family), CMV infection causes varied symptoms, commonly mosaic, vein clearing, vein banding and malformation of fruits and leaves (Zitter and Murphy, 2009).

Cucubit Aphid-borne Yellow Virus

Cucurbit Aphid-borne Yellow Virus (CABYV), a member of the genus Polerovirus in the family *Luteoviridae*, one of several viruses causing yellowing symptoms in cucumber crops. CABYV was first described in 1992 in France and early infections with CABYV may lead to a 50% yield loss in cucumber (Lecoq *et al*., 1992). CABYV multiplies in phloem tissue and is transmitted persistently from plant to plant by aphids, mainly by *Aphis gossypii* Glover and *Myzus persicae* Sulzer. It cannot be transmitted mechanically (Dogimont *et al*., 1996; Lecoq *et al*., 1992). Common symptoms in cucumber, melon, squash, and watermelon include yellowing and thickening of the older leaves. CABYV severely reduces yield in cucumber by reducing the number of fruits per plant as a result of a high percentage of flower abortions (40 and 51%, respectively) but it does not alter fruit shape or quality (Dogimont *et al*., 1996).

Zucchini Yellow Mosaic Virus

Zucchini Yellow Mosaic Virus (ZYMV), is a member of the genus Potyvirus and family *potyviridae*, and all *cucurbitaceous* crops are susceptible to ZYMV, it also infects certain non-cucurbitaceous weeds and wild cucurbits. The infection causes blistered, deformed leaves with severe mosaic symptoms besides reduction in size and stunted plant growth. Cucumber fruits get mosaic of variable intensity, dark green vein banding and discoloration. Melon fruits get discolored with hardening of flesh, seed deformation, and external cracks (Desbiez and Lecoq, 1997). Pumpkin and zucchini fruits become discolored and deformed due to knobbiness (Blua and Perring, 1989). The virus is transmitted by a wide range of aphid species of which the green peach (*Myzus persicae*) and melon (*Aphis gossypii*) aphids are the most important. The cowpea aphid (*Aphis craccivora*) has been reported to transmit the virus more efficiently than the cotton or melon aphid (Yuan and Ullman, 1996).

Papaya Ringspot Virus

Papaya Ring Spot Virus (PRSV) biotype "W" infects many cucurbit crops but does not infect non-cucurbitaceous crops (Bateson *et al*., 2002). Some wild and native cucurbit species act as infection reservoirs. Characteristic mosaic symptoms appear as profuse mottling, puckering, and deformation of crown leaves, while the symptoms do not occur on lower mature leaves. Leaf distortion and blistering have also been reported. Zucchini squash is the most susceptible to the virus, followed by watermelon and cucumber (Mansilla *et al*., 2013). Infected zucchini and pumpkin develop lumpy, distorted fruits. Watermelon fruits may develop uneven surface or typical ring spot patterns on the skin. This virus is spread by a number of aphid species including the green peach and melon aphids (Jensen, 1949).

Watermelon Mosaic Virus

Watermelon Mosaic Virus is a virus of world-wide importance in temperate and Mediterranean regions (Lecoq and Desbiez, 2008). The virus infects almost all cucurbit crops, mainly *Cucumis sativus*, *Cucurbita pepo* Linnaeus, *C. maxima* Lamarck, *and C. moschata* Duchesne (Greber, 1978), and also wild cucurbits. The diseased vines bear a typical petunia-like appearance. It represents a broader host range than most potyviruses, causing agronomic problems in important crops, mostly cucurbits. The internodal length of the shoots gets shortened, resulting in crowding of leaves that become rolled, blistered, and small in size with mild mosaic symptoms and have little effect on fruits. It produces less severe symptoms on cucurbit leaves and fruits than PRSV, ZYMV, and SqMV (Coutts, 2006).

2.7 Weed Hosts

Weeds are important in the ecology of field crops, and when crops are harvested, they often become the main hosts for plant viruses and their insect vectors, they also serve as virus reservoirs for secondary spread by insect vectors to infect field crops. (Chen *et al*., 2013). Wild plants serve as sources of inoculum and or reservoirs of viruses for crops that grow within the same field or in nearby farms (Duffus, 1971). Weeds also compete with cucumber plant for both space and nutrients. *Euphorbia hirta* Linnaeus (family *Euphorbiaceae*) and *Synedrella nodiflora* Gaertn (family *Asteraceae*) have been found to be host of CMV in south west Nigeria (Ayo-John *et al*., 2014), while *Amaranthus palmeri* S. Wats (family *Amaranthacea*) was detected as host of WMV and *Ipomea hederacea* Jacq. (family *Convolvulaceae*) as host of ZYMV in Oklahoma U.S.A (Ali *et al*., 2012).

2.9 Virus Disease Management

Management of virus diseases is difficult given the complex and dynamic nature of virus epidemics and the great ability for it to evolve over time (Acosta-Leal *et al*., 2011; Elena *et al*., 2014). For efficient and durable control of virus diseases, it is necessary to factor the genetic diversity and evolution of virus populations and have specific, fast and reliable diagnostic tools. Disease management in agriculture is based on two approaches: immunization to get resistant plants to viral infections and prophylactic measures to restrain virus dispersion (Rubio *et al*., 2020).

Some of the most common approaches for management of virus diseases are:

a) Growing resistant varieties: Planting resistant cultivars can serve as an effective control for viral diseases. The most effective and simplest method of managing viral diseases is to utilize genetic resistance. Even though resistance has been found in some cucurbit species, genetic barrier due to incompatibility among species poses a problem for transferring this resistance to other cucurbits (Zitter and Murphy, 2009).
b) Quarantine (control of borders) and sanitary certification of virus-free germplasm (seeds or asexual propagative tissues) are the first measures to prevent the introduction and emergence of new viruses into a new geographical area.
c) Rogueing is effective only if the virus incidence is low after a recent introduction or in isolated areas. Other practices consist of interrupting the virus transmission chain.
d) Insecticidal control (chemical control) Seed-borne viruses that are carried on the seed coat can be removed with chemical disinfectants such as sodium hypochlorite, trisodium phosphate and hydrochloric acid, similarly some seed-transmitted viruses can be eliminated using thermotherapy (Paylan *et al*., 2014).

e) Managing of vectors: Most plant viruses are transmitted by arthropod vectors, mainly aphids, whiteflies and thrips. Vector populations can be reduced with pesticides and natural enemies.

2.10 Virus Disease Diagnostic Methods

Plant viruses cause major losses to several agricultural crops around the world. Unlike other plant pathogens, no direct methods are available yet to manage viruses, and consequently the current measures rely on indirect or evasive tactics to manage the viral diseases. Therefore, the method for detection and identification of the virus on both the plant and the vector play a very critical role in virus disease management. Aboul-Ata *et al*. (2011) stated that methods for detection and identification of viruses are crucial in management of virus diseases. The early detection of plant viruses constitutes one of the main ways of managing these diseases. Several methods have been developed to detect plant viruses, such as microscopical observation, serological techniques, molecular methods e.t.c (Makkouk and Kumari, 2006).

2.10.1 Enzyme linked immunosorbent assay

The Enzyme-Linked Immunosorbant Assay (ELISA) is a simple and sensitive method for detection and quantification of the virus level in plants (Clark and Adams, 1977). Fluctuations in virus concentrations are known to occur throughout the crop cycle, and plants that have low virus content may equally test negative for a virus if assayed when the virus titer is lowest (Conci *et al*., 2002). The most widespread technique for virus identification is DAS-ELISA because this process combines economical reagents and versatility, and accommodates large-scale testing. Unfortunately, if samples are analysed when virus concentration is quite low, DAS-ELISA cannot differentiate between healthy and infected plants (Dovas *et al*., 2001). Therefore, for accurate detection, the optimum time for sampling must be determined based on virus titer.

Double antibody sandwich- enzyme linked immunosorbent assay (DAS-ELISA) method is best suited for the detection of the presence of CMV, ZYMV and WMV.

2.10.2 Tissue blot immunoassay (TBIA)

The principles of Tissue Blot Immunoassay (TBIA) is the same with that of Enzyme Linked Immunosorbent Assay (ELISA) to which antibody is applied, TBIA has the same reliability as ELISA to detect plant viruses (Hancevic *et al*., 2012). One of the major difference is that polystyrene plate is used as the platform of ELISA, whereas TBIA is performed on nitrocellulose and nylon membranes. Like ELISA, TBIA also make use of a specific antibody to get rid of false positive and also needs large amount of virus concentration to reduce false negative. However, TBIA has great benefits over ELISA in terms of detection time, cost, sensitivity and convenience, it has been applied for diagnosis of a number of viral diseases caused by *Bamboo mosaic virus* (BoMV), *Bean yellow mosaic virus* (BYMV), *Citrus tristeza virus* (CTV), *Cymbidium mosaic virus* (CyMV), *Papaya ringspot virus* (PRSV), *Sweet potato feathery mottle virus* (SPFMV), and *Tomato spotted wilt virus* (TSWV) (Makkouk and Kumari, 2006, Hancevic *et al*., 2012).

2.10.3 Dot-blot hybridization

This technique involves the direct application of nucleic acid solution to a solid support, such as nitrocellulose or nylon membranes and direction with appropriate probes. The use of non-radioactive precursors to label nucleic acids has made the molecular hybridization technique more accessible and is being used in more virus-host combinations (Pallás *et al*., 1998).

2.10.4 Polymerase chain reaction (PCR)

Polymerase Chain Reaction is a scientific technique used to amplify, or create millions of identical copies of a particular DNA sequence within a tiny reaction tube. Prior to the initiation

of each new round for DNA amplification, the DNA is denatured, two sets of oligonucleotides (called primers) anneal to the denatured complementary strand. Then, primers lead DNA synthesis by the DNA polymerase. All reactions occur sequentially in template dependent manner. Through this, the target sequences of interesting DNA are exponentially amplified (Saiki *et al*., 1985, 1988). Polymerase chain reaction has been used as one of the core techniques to molecular biology based-researches in a many of applications such as cloning, gene manipulation, gene expression analysis, genotyping, sequencing, and mutagenesis. In addition, PCR has also been used as a diagnostic tool to detect virus diseases (Makkouk and Kumari, 2006).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Determination of Incidence and Distribution of *Cucumber Mosaic Virus, Watermelon Mosaic Virus* and *Zucchini Yellow Mosaic Virus* in Cucumber in Kaduna and Kano States

3.1.1 Field surveys and sampling of cucumber fields

Survey was conducted during the 2020 dry (February-April) and rainy (August-October) seasons to determine the occurrence of *Cucumber mosaic virus, Watermelon mosaic virus and Zucchini yellow mosaic virus* on fields in Sabon Gari, Giwa and Kudan Local Government Areas (LGAs) of Kaduna State and Kura, Minjibir and Bunkure Local Government Areas of Kano state. Three farms in each LGA were surveyed per State**.**

In each field, a 2 × 2 m^2 sized-quadrats was set up at four corners of the field with one in the middle, there (3) cucumber leaf samples were collected from each quadrat (2 symptomatic and 1 asymptomatic), giving a total of 15 samples from each farm, and 45 samples for each LGA and 135 samples for each State. The number of infected and total number of plants in the quadrat were recorded, both during dry (February-April) and rainy(August-October) seasons.

Leaf samples collected were, carefully labeled, stored in sample bottles with calcium chloride and then transported to the Virology Laboratory of the Department of Crop Protection, Ahmadu Bello University (A.B.U), Zaria for diagnosis. Collected weed samples were identified at the Herbarium of the Department of Botany, Faculty of Life Sciences, Ahmadu Bello University, (ABU) Zaria. The disease incidence was calculated by dividing the number of diseased plants by the total number of plants examined for visual observation as stated by Chaube & Pundhir. (2005).

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

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 include: name of LGA and the co-ordinates of the locations which was determined with the aid of Global Positioning System (GPS). A questionnaire (Appendix I) 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, cucumber variety, source of seed, symptoms observed on cucumber plants and weed species, this information's were recorded.

3.1.2 Laboratory analyses of samples

Double Antibody Sandwich Enzyme Linked Immunosorbent Assay (DAS-ELISA) was used to determine the presence of *Cucumber mosaic virus Watermelon mosaic virus* and *Zucchini yellow mosaic virus* in the leaf samples collected from Kaduna and Kano states. The technique was used to test the symptomatic and asymptomatic cucumber and weed leaf samples for the presence of the viruses using antisera purchased from German Collection of Microorganisms and Cell Cultures GmbH, Germany.

ELISA 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 2hrs.

Samples were extracted in sample extraction buffer by grinding leaves in extraction buffer 1:10 (w/v). 200 micro litre (µl) aliquots of the test samples were added to each well of the antibody

coated ELISA plates and then incubated in a refrigerator (4°C) overnight. The plates were washed 3 times with PBS-Tween as described earlier. Two hundred micro litre (µl) monoclonal antibody diluted at 1:1000 in conjugate buffer was dispensed into each well. The plates were covered and incubated at 37 °C for 2 hours and washed as before. P-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 (Biochrom EZ Read 400) at $A_{405nm.}$ ELISA values at least twice the values of the negative control were rated positive as reported by Kumar (2009).

3.2 Determination of Weed Host of CMV, WMV and ZYMV

Same procedure was followed as 3.1.2 except that, five weed samples within and around the cucumber farms (up to 5 m distance) were randomly sampled for both state making a total of 180 weed samples in both states and seasons. A total of 15 weed samples per LGA and 45 weed samples for each State. The collected weed samples were identified at the Herbarium of the Department of Botany, Faculty of Life Sciences, Ahmadu Bello University, Zaria.

3.3 Data Analysis

Data collected on the virus disease incidence from all six Local Government Areas were analysed using descriptive statistics (Bar Chart) on Microsoft Excel 17^{th} edition.

CHAPTER FOUR

4.0 RESULTS

4.1 Symptoms of Cucumber Viruses in the field

The different symptoms observed on the fields visited (Plate 1) include mosaic patterns of light and dark green, chlorotic stripes and necrosis. Other symptoms observed include stunting, malformation and mottling of leaves. These symptoms are shown on Plate I. Samples that tested positive for *Cucumber mosaic virus, Watermelon mosaic virus* and *Zucchini yellow mosaic virus* had one or more of the symptoms mentioned above and the viruses occurred in single and mixed infections.

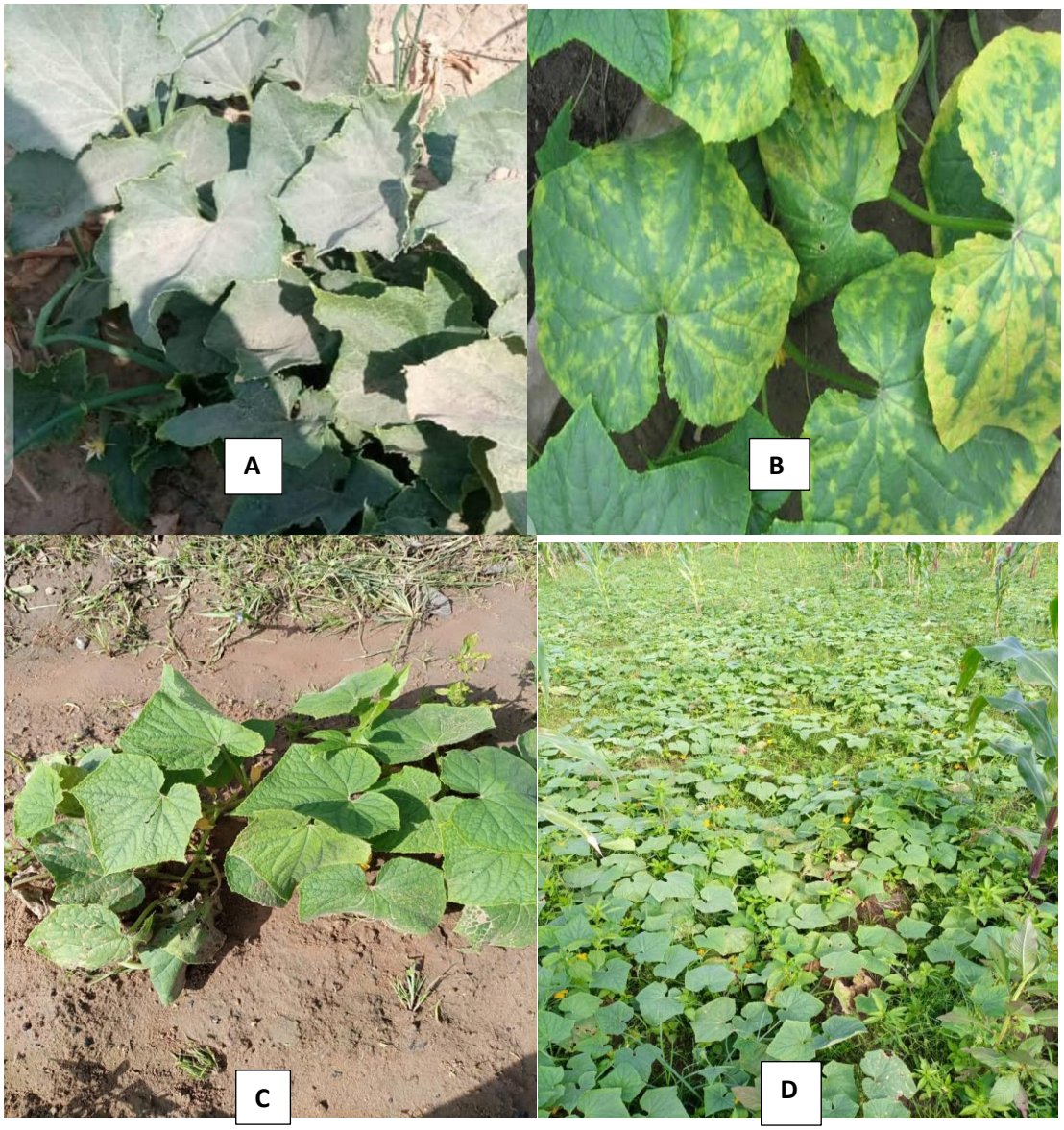

Plate I: Cucumber leaves showing symptoms of *Zucchini yellow mosaic virus* (B) *Cucumber mosaic virus* (C) and *Watermelon mosaic virus* (D) compared with healthy plant (A)

4.1.1 Incidence of the viruses on cucumber in Kaduna state during the 2020 dry and rainy seasons

Out of the forty-five samples tested in Sabon Gari LGA during the dry season, ELISA results showed that nine were positive. *Cucumber Mosaic Virus* (CMV) had the highest incidence of 20% followed by *Watermelon Mosaic Virus* (WMV) with 4.40% incidence and *Zucchini Yellow Mosaic Virus* (ZYMV) with 2.2 % incidence. The CMV+WMV mixed infection had incidence of (6.67%), while CMV+ZYMV mixed infection had an incidence of 13.34% as shown in Figure 1 and Table 1.

In Kudan LGA forty-five samples were tested out of which four were positive, CMV had the highest incidence of 8.90 % followed by WMV with 4.40 %, while ZYMV was not detected in any of the samples in this LGA. CMV+WMV was the only mixed infection detected with 6.67 % incidence in this area (Figure 1 and Table 2).

In Giwa LGA, only two out of the forty-five samples tested was positive. CMV and WMV each had an incidence rate of 2.22 % while ZYMV was not detected (Figure 1). In Sabon Gari LGA, ELISA results showed that out of the forty-five samples tested during the rainy season, fifteen were positive. CMV had the incidence rate of 33.33 % while WMV had an incidence rate of 13.33 %. CMV + WMV had an incidence of 6.67 % (Figure 2). Five out of the forty-five samples tested in Kudan LGA were positive. CMV had an incidence rate of 8.89 %, followed by ZYMV with an incidence of 6.67 %, while WMV was not detected in the Local Government during the rainy season. Mixed infection of CMV+ZYMV had an incidence rate of 6.67 % (Figure 2). In Giwa LGA, nine samples were positive out of the forty-five tested, ZYMV had the highest incidence of 13.33 %, while CMV had an incidence of 6.69 % as shown in Figure 2.

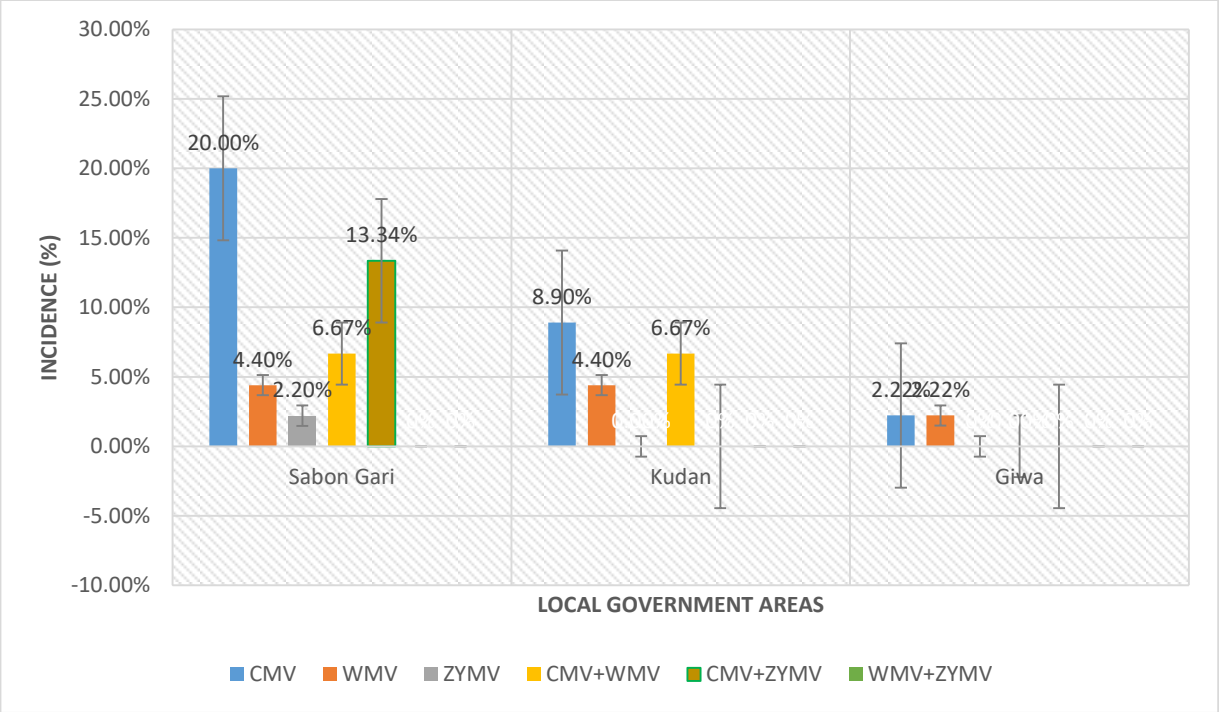

Figure 1: Incidence of viruses on Cucumber in Kaduna State during the 2020 dry season (Feb-Apr)

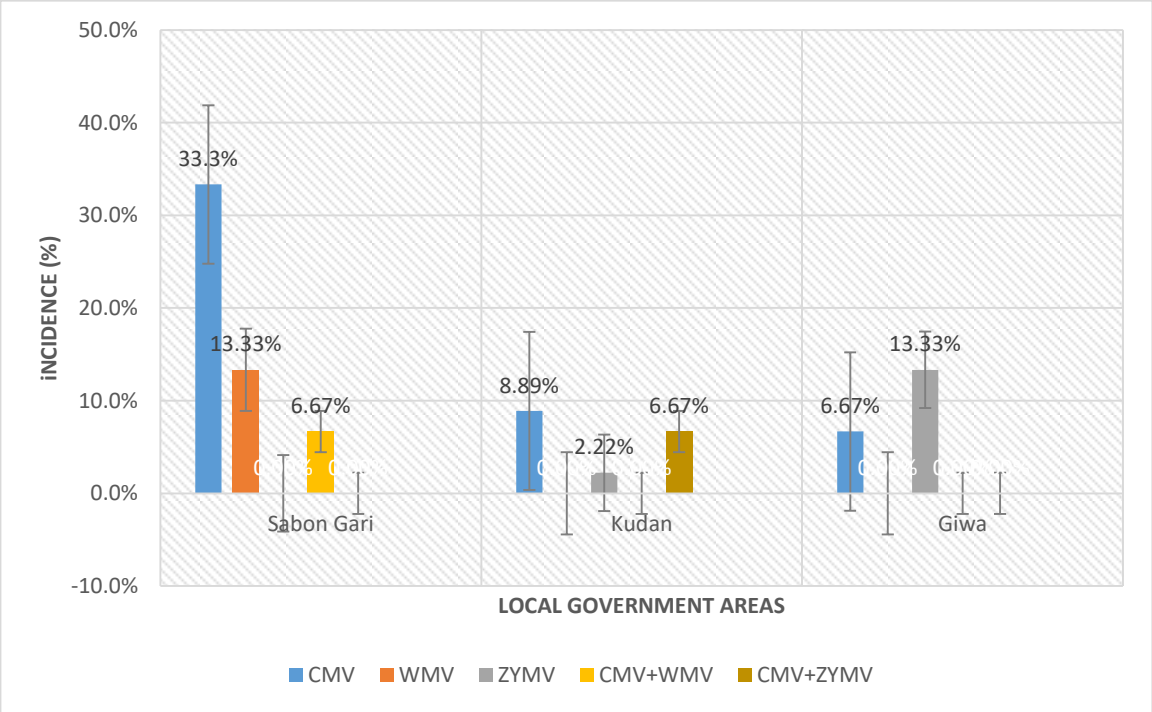

Figure 2: Incidence of viruses on Cucumber in Kaduna State during the 2020 rainy season (Aug-Oct)

4.1.2 Incidence of the viruses on cucumber in Kano state during the dry and rainy seasons

The ELISA results for the cucumber leaf samples collected in Kura LGA showed that out of Forty-five samples tested during the dry season, nine samples were positive, and WMV had the highest incidence of 15.56% followed by CMV with 4.45%. *Zucchini Yellow Mosaic virus* or mixed infections was not present as shown in Figure 3

In Bunkure LGA, ELISA shows that out of the forty-five samples tested five were positive, CMV and WMV had the same incidence of 4.42%, followed by ZYMV with an incidence rate of 2.22%. Mixed infection of CMV+WMV was present with an incidence rate of 13.33% (Figure 3). In Minjibir LGA out of forty-five samples that were tested ten were positive. WMV had the highest incidence of 15.56% followed by CMV with 6.66% incidence and ZYMV was not detected. Mixed infection of CMV+WMV was detected at an incidence rate of 6.67% as shown in Figure 3.

During the rainy season in Kura LGA, forty-five samples were tested, thirteen were positive, CMV had the highest incidence of 26.68%, followed by WMV with an incidence of 2.22%. ZYMV was not detected in this LGA as shown in Figure 4. In Bunkure LGA, forty-five samples were also tested and fifteen were positive. CMV had the highest incidence of 24.45% followed by ZYMV with 8.89%. WMV was not detected in this LGA (Figure 4). While in Minjibir LGA, forty-five samples were also tested and five were positive. CMV and ZYMV had an incidence of 4.45% respectively, while WMV had an incidence of 2.22% (Figure 4).

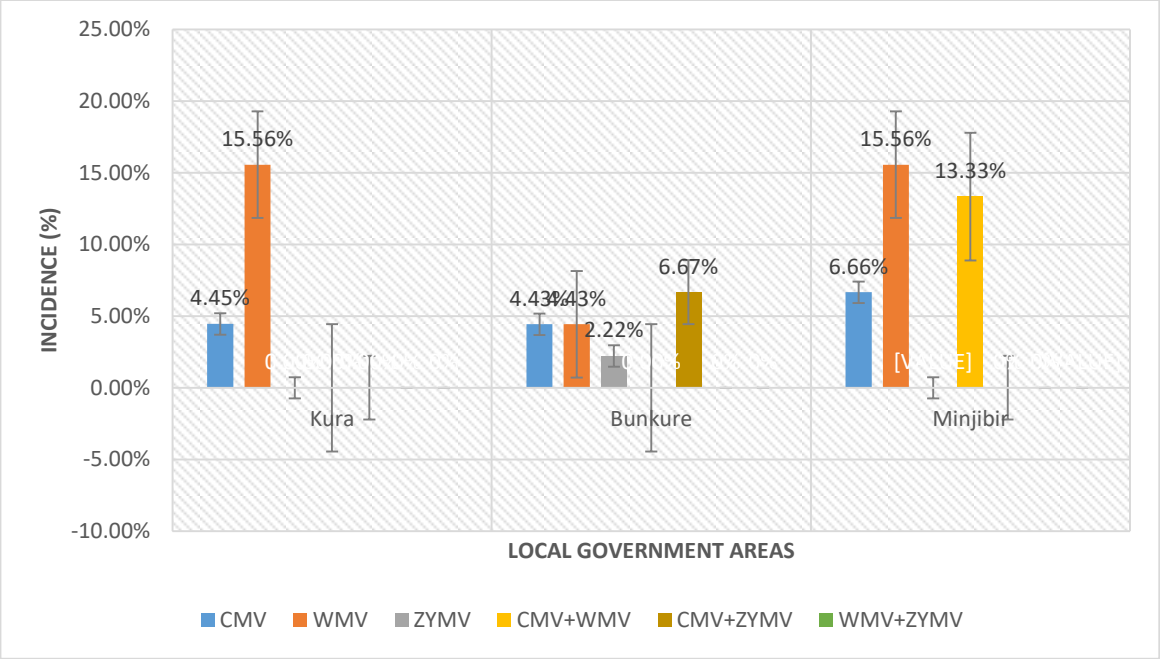

Figure 3: Incidence of viruses on Cucumber in Kano State during the 2020 dry season (Feb-Apr)

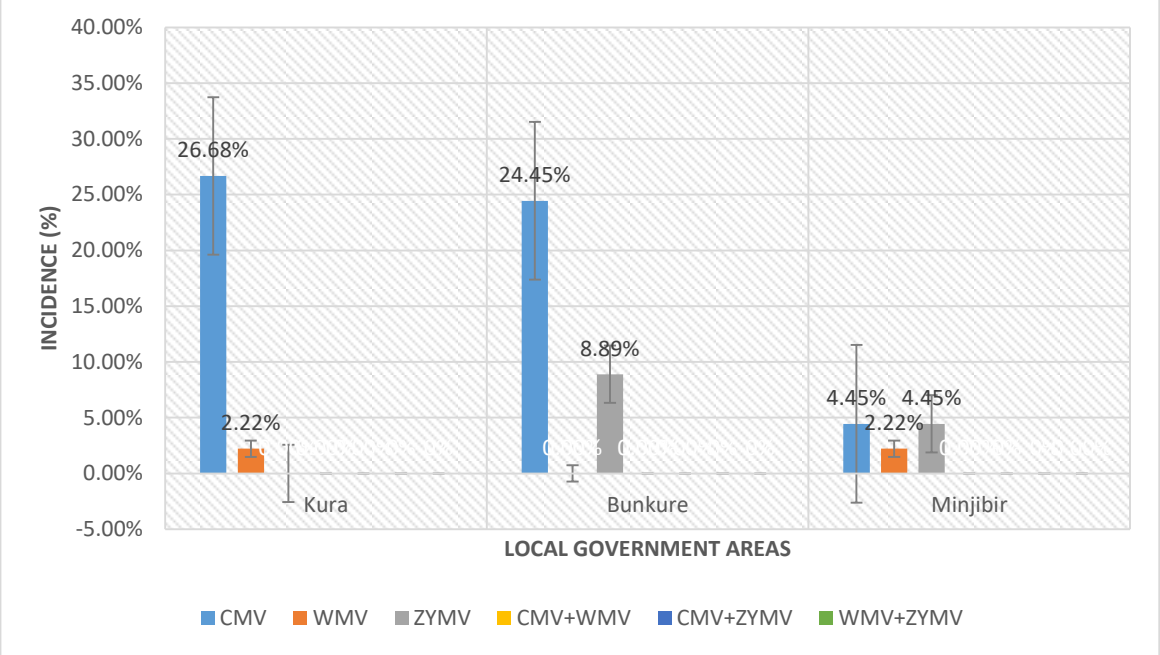

Figure 4: Incidence of viruses on Cucumber in Kano State during the 2020 rainy season (Aug-Oct)

4.1.3 Incidence of viruses on cucumber in kaduna and kano states during the 2020 dry and rainy seasons

During the dry season in Kaduna state, 135 samples were tested out of which 14 samples were positive. CMV had the highest incidence of 10.37%, followed by WMV which had an incidence rate of 3.67%, ZYMV had an incidence of 0.73%. Mixed infection of CMV+WMV and CMV+ZYMV each had an incidence rate of 4.45% respectively as shown in Figure 5.

In Kano State ELISA results showed that 24 out of the 135 samples tested were positive during the dry season were positive. WMV had the highest incidence rate of 11.85% followed by CMV which had an incidence of 5.18%. ZYMV had an incidence rate of 0.74% and mixed infection of CMV+WMV had an incidence of 4.44% while CMV+ZYMV had an incidence rate of 2.33% (Figure 5).

In the rainy season, 29 samples were detected to be positive out of the 135 samples tested in Kaduna State. CMV had the highest incidence of 16.28%, followed by ZYMV which had an incidence of 5.18%. WMV had an incidence of 4.44%, while mixed infection of CMV+WMV and CMV+ZYMV both had an incidence of 2.22% respectively (Figure 6).

During the rainy season in Kano State, 135 samples were also tested using ELISA and 35 tested positive. CMV had the highest incidence of 18.53%, while ZYMV had an incidence of 4.44% and WMV with an incidence rate of 1.48% as shown in Figure 6.

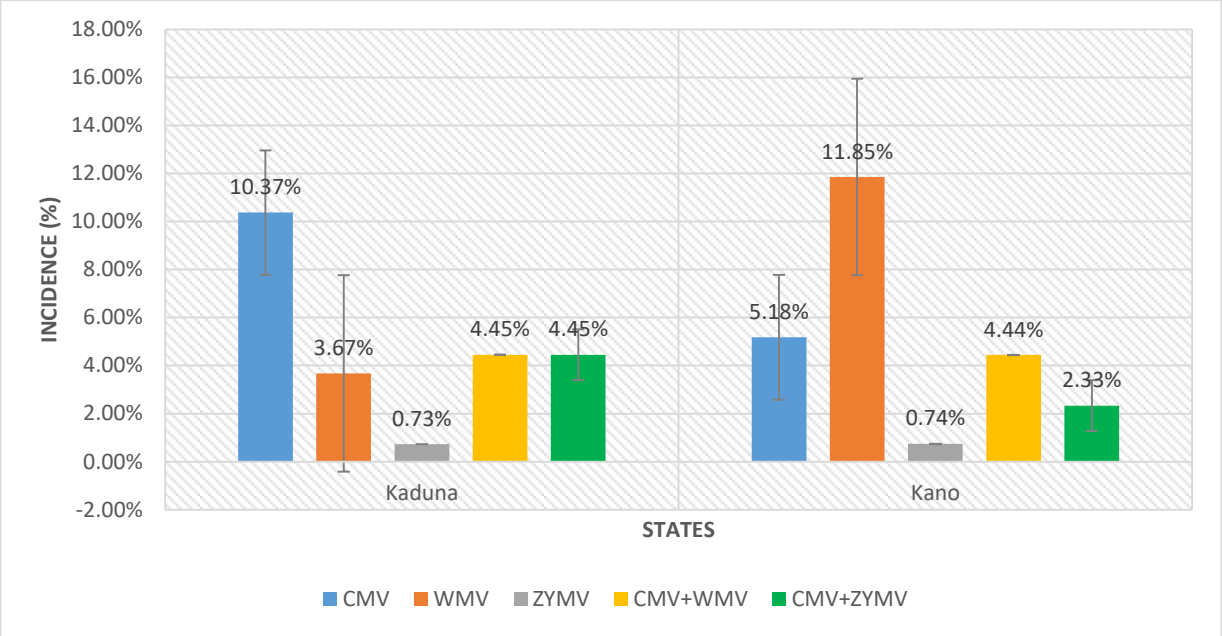

Figure 5: Incidence of Viruses on Cucumber in Kaduna and Kano States during the 2020 dry season (Feb-Apr)

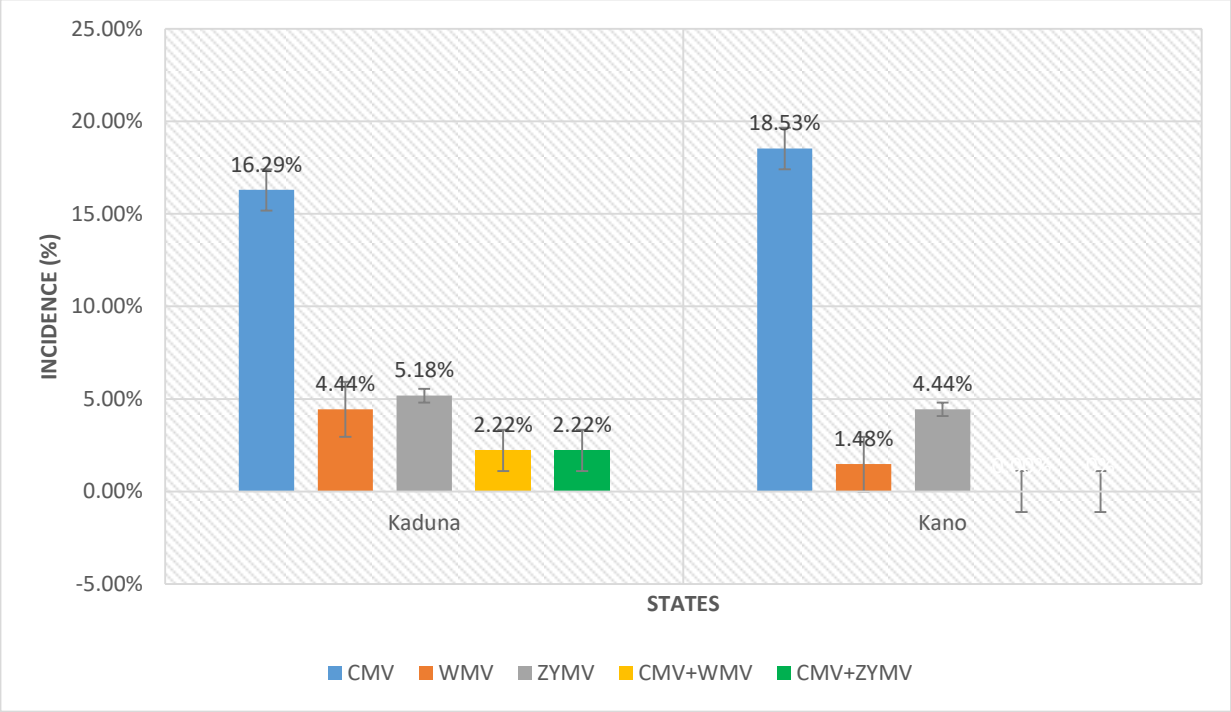

Figure 6: Incidence of Viruses on Cucumber in Kaduna and Kano States during the 2020 rainy season (Aug-Oct)

4.1.4 Distribution of the viruses in kaduna and kano states during the 2020 rainy and rry seasons

The distribution of the viruses infecting Cucumber in Kaduna State (Table 1 and 2) varies moderately between farms and considerably between seasons. *Cucumber mosaic virus* was detected in all three LGAs during the dry and rainy seasons and only Sabon Gari had all three viruses present (Table 1 and Figure 7). *Watermelon mosaic virus* was also detected in all three LGAs during the dry season as shown in Table 1, but just in Bomo and Hayin Mai Dakali (Sabon Gari LGA) during the rainy season (Table 2). *Zucchini yellow mosaic virus* was not detected in Sabon Gari during the rainy season (Table 2), and was also only detected in Bomo Sabon Gari LGA during the dry season. Mixed infection of *Cucumber mosaic virus + Watermelon mosaic virus* was detected in Shika Sabon Gari LGA during the both seasons. In Kudan LGA mixed infection of CMV+WMV was detected in Hunkuyi (Table 1) during the dry season and mixed infection of WMV+ZYMV was detected in Mai Gaya Musawa during the rainy season. CMV+ZYMV mixed infection was also detected in Bomo Sabon Gari LGA during the dry season.

Cucumber mosaic virus was detected in all three LGAs in Kano during both seasons (Tables 3 and 4), WMV was also detected in Kura and Minjibir LGAs in both seasons, but was not detected in Bunkure during the rainy season (Table 4). ZYMV was detected in Zango (Bunkure LGA) during the dry season as shown in Table 3, and was not detected in Kura LGA during the rainy season. Mixed of CMV+WMV was detected in Minjibir and CMV+ZYMV in Bunkure during the dry season (Table 3).

Table 1: Distribution of *Cucumber Mosaic Virus*, *Watermelon Mosaic Virus* and *Zucchini Yellow Mosaic Virus* on Cucumber in Kaduna State during the dry season (Feb-April)

LGA	Location	Number of cucumber samples Tested	Number of Positive Samples						
			Single infections			Mixed infections			
			CMV	WMV	ZYMV	CMV+WMV	CMV+ZYMV	WMV+ZYMV	CMV+WMV+ ZYMV
Sabon Gari	Bomo	15	3+	-	1+	-	1+	-	-
	Hayin Mallam	15	5+	1+	-	-	-	-	-
	Shika	15	1+	1+	-	+1	-	-	-
Kudan	Jaja	15	3+	-	-	-	-	-	-
	Jaja	15	-	-	-	-	-	-	-
	Hunkuyi	15	1+	2+	-	+1	-	-	-
Giwa	Dan Mahawiya	15	-	-	-	-	-	-	-
	Giwa Sabuwa	15	-	-	-	-	-	-	-
	Rafin	15	1+	1+	-	-	-	-	-
Total		135	14	5	1	2	1	-	-

CMV=*Cucumber Mosaic Virus*, WMV= *Watermelon Mosaic Virus*, ZYMV= *Zucchini Yellow Mosaics Virus*, - = Not detected, + = detected

Table 3: Distribution of *Cucumber Mosaic Virus* , *Watermelon Mosaic Virus* and *Zucchini Yellow Mosaic Virus* on Cucumber in Kano State during the

LGA	Location	Number of cucumber samples Tested	Number of Positive Samples						
			Single infections			Mixed infections			
			CMV	WMV	ZYMV	CMV+WMV	CMV+ZYMV	WMV+ZYMV	CMV+WMV+ ZYMV
Sabon Gari	Bomo	15	3+	4+	-	1+	-	-	-
	Hayin Mai Dakali	15	8+	2+	-	1+	-	-	-
	Angwan Bisa	15	4+	-	-	-	-	-	-
Kudan	Jaja	15	2+	-	-	-	-	-	-
	Mai Gaya Musawa	15	1+	-	1+	-	-	1+	-
	Hunkuyi	15	1+	-	-	-	-	-	-
Giwa	Panhauya	15	1+	-	5+	-	-	-	-
	Abadawa	15	1+	-	-	-	-	-	-
	Angwan Makama	15	1+	-	1+	-	-	-	-
Total		135	22	6	7	2	-	1	-

CMV=*Cucumber Mosaic Virus*, WMV= *Watermelon Mosaic Virus*, ZYMV= *Zucchini Yellow Mosaics Virus*, - = Not detected, + = detected

Table 4: Distribution of *Cucumber Mosaic Virus*, *Watermelon Mosaic Virus* and *Zucchini Yellow Mosaic Virus* on Cucumber in Kano State during the wet season (Aug-Oct)

dry season (Feb-Apr)

LGA	Location	Number of cucumber samples Tested	Number of Positive Samples						
			Single infections			Mixed infections			
			CMV	WMV	ZYMV	CMV+WMV	CMV+ZYMV	WMV+ZYMV	CMV+WMV+ ZYMV
Kura	Kadani	15	-	7+	-	-	-	-	-
	Dauni	15	1+	-	-	-	-	-	-
	Gamadam	15	1+	-	-	+1	-	-	-
Bunkure	Gaba da Gari	15	-	2+	-	-	-	-	-
	Zango	15	2+	-	1+	-	-	-	-
	Bunkure Gabbas	15	-	-	-	-	+1	-	-
Minjibir	Farin Gada	15	2+	7+	-	-	-	-	-
	Wasai	15	-	-	-	-	-	-	-
	Marke	15	1+	-	-	-	-	-	-
Total		135	7	5	1	-	1	-	-

CMV=*Cucumber Mosaic Virus*, WMV= *Watermelon Mosaic Virus*, ZYMV= *Zucchini Yellow Mosaics Virus*, - = Not detected, + = detected

		of cucumber samples Tested	Single infections			Mixed infections			
			CMV	WMV	ZYMV	CMV+WMV	CMV+ZYMV	WMV+ZYMV	CMV+WMV+ ZYMV
Kura	Bomo	15	1+	1+	-	-	-	-	-
	Hayin Mallam	15	4+	-	-	-	-	-	-
	Shika	15	7+	-	-	-	-	-	-
Bunkure	Jaja	15	2+	-	-	-	-	-	-
	Jaja	15	4+	-	1+	-	-	-	-
	Hunkuyi	15	5+	-	3+	-	-	-	-
Minjibir	Hayin Gada	15	1+	-	-	-	-	-	-
	Abadawa	15	-	-	1+	-	-	-	-
	Angwan Makama	15	1+	1+	1+	-	-	-	-
Total		135	25	2	6	-	-	-	-

CMV=*Cucumber Mosaic Virus*, WMV= *Watermelon Mosaic Virus*, ZYMV= *Zucchini Yellow Mosaics Virus*, - = Not detected, + = detected

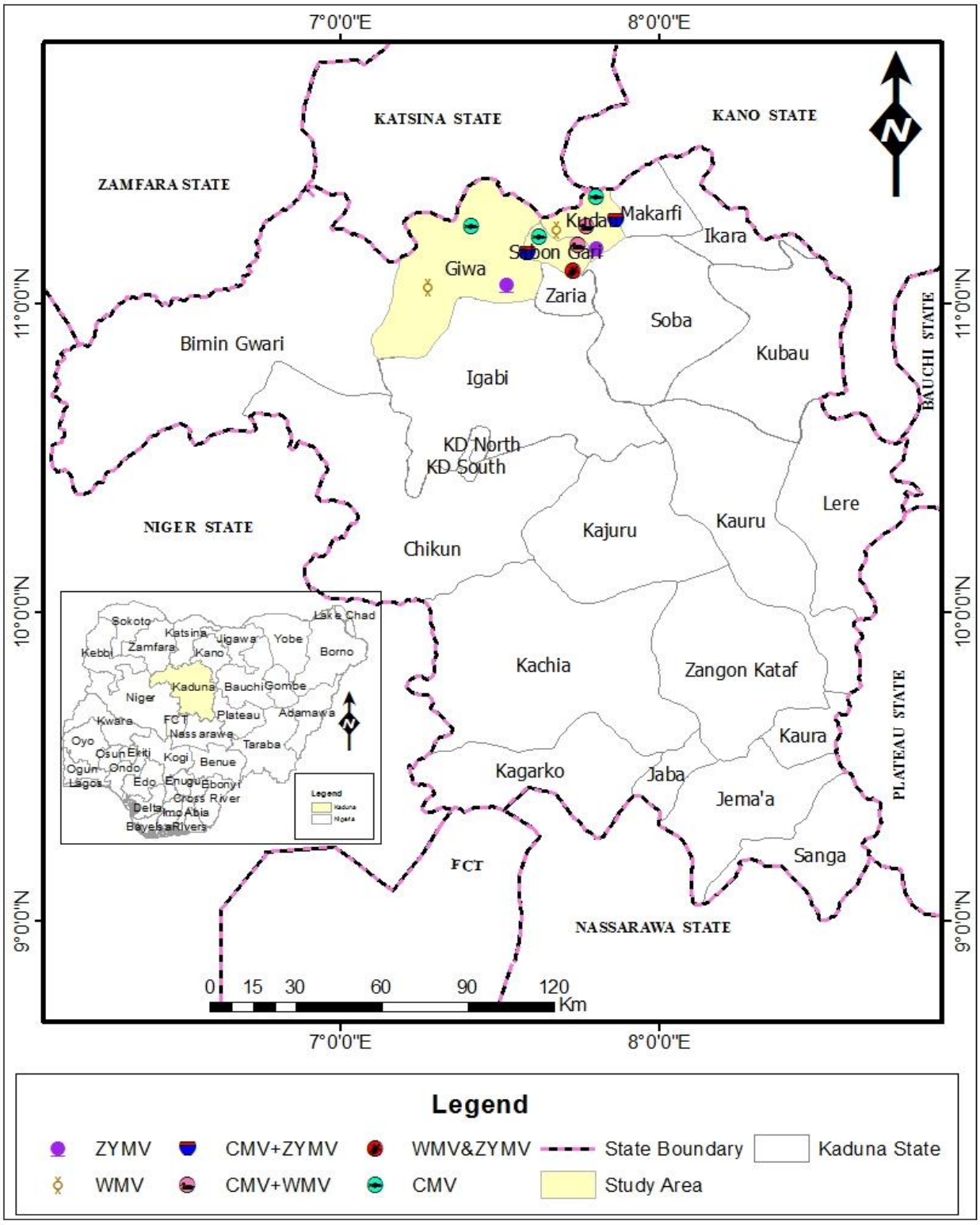

Figure 7: *Map of Kaduna State Showing the Study Area with the spatial location of Virus detection.*

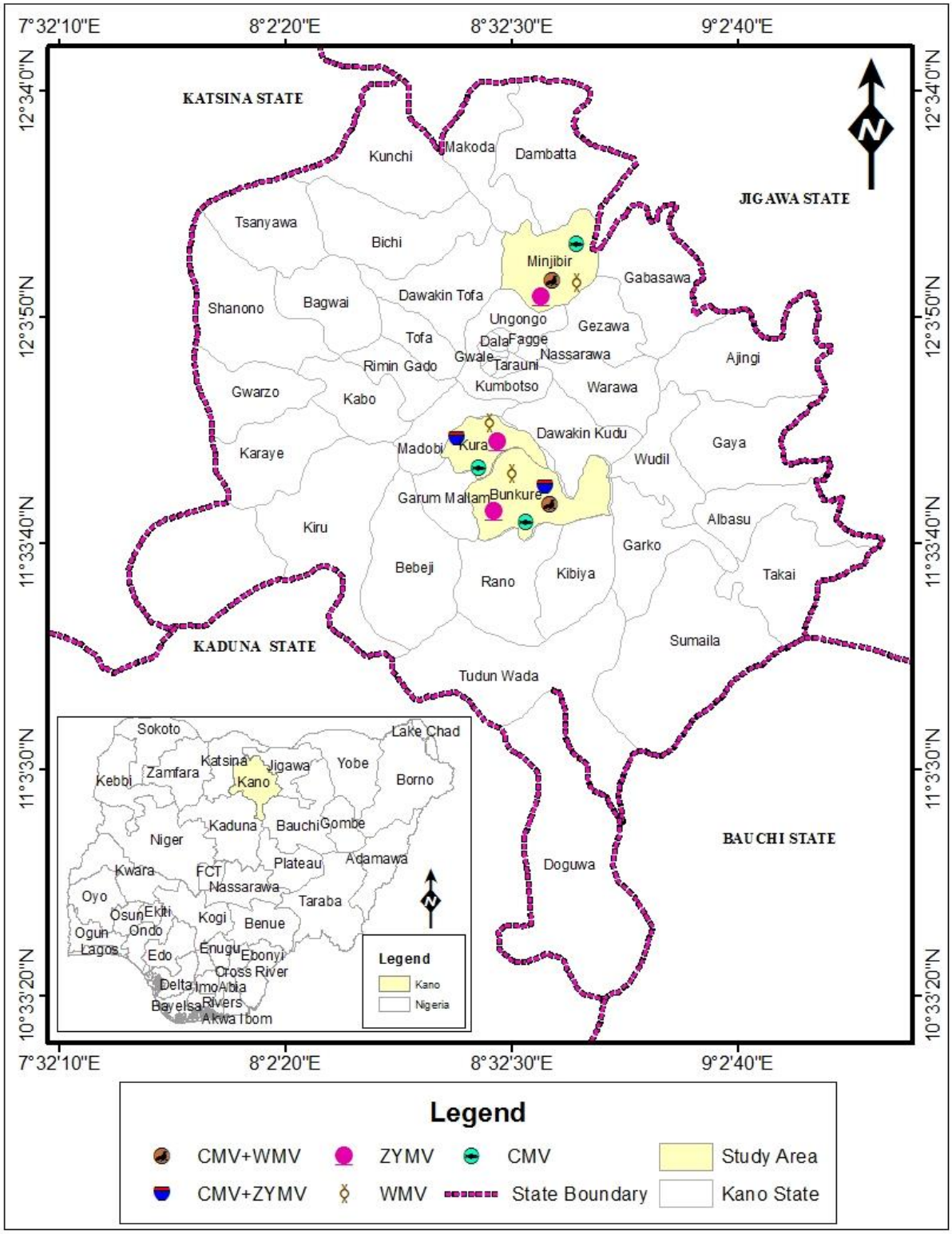

Figure 8: *Map of Kano State Showing the Study Area with the spatial location of Virus detection.*

4.2 Weed Hosts of Cucumber Viruses in Kaduna and Kano State

Ninety weed samples were collected in both seasons. Forty-five (45) weed samples were collected in the 2020 dry season and 45 weed samples as well in the wet season. They were identified and found to belong to 14 plant families. During the rainy season survey in Kaduna State, the weeds that tested positive for CMV and acted as reservoirs were identified as *Portulace oleracea, Commelina coelesis*. During the dry season *Euphorbia indica,* and *Waltheria indica* tested positive for CMV, only *Euphorbia indica* tested positive for WMV in the dry season (Table 5).

In Kano State, *Chenopodium album* belonging to the family *Amaranthaceae, Portulaca oleracea* of the family *Portulacaceae* and *Ageratum houstonianum* from the family *Astersceae* tested positive for CMV in both seasons while *Alternanthera brasiliana* from *Amaranthaceae* family also tested positive for WMV in both seasons making them alternative host of CMV and WMV as shown in Table 6.

Table 5: Alternative Hosts of *Cucumber mosaic virus*, *Watermelon mosaic virus* and *Zucchini yellow mosaic virus* in Kaduna State in 2020

Weed species	CMV		WMV		ZYMV	
	Wet	Dry	Wet	Dry	Wet	Dry
Amaranthaceae	-					
Alternanthera sessilis	-	-	-	-	-	-
Chenopodium album	-	-	-	-	-	-
Alternanthera brasiliana	-	-	-	-	-	-
Chenopodium polyspermum	-	-	-	-	-	-
Amaranthus spinosis	-	-	-	-	-	-
Portulacaceae						
Portulaca oleracea	+	-	-	-	-	-
Portulaca oleracea	-	-	-	-	-	-
Turneracea						
Turnera ulmifolia	-	-	-	-	-	-
Euphorbiaceae						
Euphorbia hirta	-	-	-	-	-	-
Euphorbia indica	-	+	-	+	-	-
Rubiaceae						
Mitracarpus hitra	-	-	-	-	-	-
Lamiaceae						
Ocimum minimum	-	-	-	-	-	-
Malvaceae						
Waltheria indica	-	+	-	-	-	-
Poaceae						
Arthraxon hispidus	-	-	-	-	-	-
Cynodon dactylon	-	-	-	-	-	-
Asteraceae						
Ageratum conyzoides	-	-	-	-	-	-
Synedrella nodiflora	-	-	-	-	-	-
Commelinaceae	-	-	-	-	-	-
Commelina coelesis	+	-	-	-	-	-

CMV= *Cucumber Mosaic Virus*, WMV= *Watermelon Mosaic Virus*, ZYMV= *Zucchini Yellow Mosaic Virus*, - = Not detected, + = Detected.

Table 6: Alternative hosts of the *Cucumber mosaic virus, Watermelon mosaic virus* and *Zucchini yellow mosaic virus* in Kano State in 2020

Weed species	CMV		WMV		ZYMV	
	Wet	Dry	Wet	Dry	Wet	Dry
Amaranthaceae						
Alternanthera sessilis	-	-	-	-	-	-
Chenopodium album	+	+	-	-	-	-
Alternanthera brasiliana	-	-	+	+	-	-
Chenopodium murale	-	-	+	-	-	-
Gomphrena celosioides	-	-	-	-	-	-
Brassicaceae						
Brassica napus	-	-	-	-	-	-
Rubiaceae						
Mitracarpus hitrus	+	-	-	-	-	-
Onagraceae						
Epilobium hirsutum	-	+	-	-	-	-
Thymelaeaceae						
Daphne gnidium	-	-	-	-	-	-
Fabaceae						
Waltheria indica	-	+	-	+	-	-
Portulaceae						
Portulaca umbraticola	-	-	-	-	-	-
Portulaca oleracea	+	+	-	-	-	-
Poaceae						
Arthraxon hispidus	-	+	-	-	-	-
Cynodon dactylon	-	-	-	-	-	-
Asteraceae						
Ageratum houstonianum	+	+	-	-	-	-
Synedrella nodiflora	-	-	-	-	-	+
Galinsoga parviflora	-	-	-	-	-	-
Galinsoga quadriradiata	-	-	-	-	-	-
Acanthospermum hisidum	-	-	-	-	-	-
Xanthium spinosum	-	-	-	-	-	-

CMV= *Cucumber Mosaic Virus*, WMV= *Watermelon Mosaic Virus*, ZYMV= *Zucchini Yellow Mosaic Virus*, - = Not detected, + = Detected.

CHAPTER FIVE

5.0 DISCUSSION

5.1 Determination of the Incidence and Distribution of *Cucumber Mosaic Virus, Watermelon Mosaic Virus* and *Zucchini Yellow Mosaic Virus* in Kaduna and Kano States

The incidence, distribution and weed hosts of the three cucumber viruses (*Cucumber mosaic virus, Watermelon mosaic virus* and *Zucchini yellow mosaic virus*) in Kaduna and Kano States were established in this study. Cucumber and weed species (*Chonopodium album,Portulaca oleracea, Alternanthera brasiliana, Ageratum houstonianum*) were found to harbour CMV, WMV and ZYMV either singly or as a mixed infection. The symptoms observed on infected plants which included mosaic patterns, yellow streaking, leaf deformation and stunting have been associated with CMV, WMV and ZYMV in Nigeria (Ayo-John *et al*., 2014), Cote d'Ivoire (Kone *et al*., 2017), Syria (Chikh-Ali *et al*., 2019), Mali (Knierim *et al*., 2014), and Korea (Kim *et al*., 2010). This record gives one of the first surveys on virus diseases of cucumber crop in Northwest Nigeria, where it is fast gaining preference by both farmers and consumers because of its health benefits. Virus diseases were observed in all farms surveyed and the disease incidences in the locations surveyed ranged from 2 to 30%.

After serological tests were conducted it was confirmed that the causal agents of these symptoms were the viruses detected. Some of the samples with virus-like symptoms tested negative for all viruses studied. This could have been as a result of other viruses present, and whose antisera were not used in this study or as a result of other biotic or abiotic agents. The virus incidence varied across the two states, the Local Government areas and seasons among the farms in the study areas.

Serological analysis of the leaf samples showed that *Cucumber mosaic virus* was the most prevalent virus both in the cucumber crop and weeds and in the two states surveyed in North

west Nigeria. *Cucumber mosaic virus* was detected in all six LGA's in both States during the dry and rainy season. Sabon Gari recorded the highest incidence, followed by Kura during the rainy season, this finding is in contradiction to the finding of Kone *et al*. (2017), who reported higher prevalence of CMV in dry season than in rainy season in Cote d'Ivoire. This could have also been because of poor weed management measures, as farms in Sabon Gari had the most weed infestation (Appendix IV). It could have also been caused by insect pest, as the Sabon Gari had the most Aphid infestation (Appendix IV). Watt *et al*. (2020) reported that Aphids introduce virus particle directly into the host plant during feeding activities and aphids have been reported to be vectors of CMV.

Watermelon mosaic virus was detected in all of the LGAs during the dry season in both Kaduna and Kano States, while during the dry season (Aug-Oct) it was only detected in Sabon Gari as shown in Figure 3. The high prevalence of the disease during the dry season (Feb-Apr) could be as a results of Aphids which were also observed on the farms as the temperature was high when samples were taken which are favourable for vector development and spread (Appendix III), which results in increased disease development as also reported by Kone *et al*. (2017). The high prevalence of the disease could also be due to the farmers poor agronomic practice such as poor farm sanitation (no rogueing, and weedy fields) as shown in Appendix III (Hull, 2009). WMV is one of the most widespread Potyviruses with the broadest host range infecting cucumber (Ali and Natsuaki, 2007). WMV was not detected in Kudan and Giwa LGAs in Kaduna State and in Bunkure in Ksano State during the raining season and its incidence were low in LGAs where it was detected, this could have been as a result of the rains, as samples were taken at the peak of the rainy season when the primary vector was not readily available. This finding agrees with

Kone *et al*., (2017) who reported low incidence rates during the rainy season as high rainfall negatively affect vector populations and their mobility preventing the spread of the viral disease.

Zucchini yellow mosaic virus was detected in just Sabon Gari LGA (Kaduna) and Bunkure LGA (Kano) during the dry season. ZYMV was found to be at the highest during the rainy season in Giwa LGA followed by Bunkure LGA although the incidence where low, this contradicts the work of Clarke *et al*. (2020) which reported that ZYMV is more prevalent during the dry season and found to be low in the rainy season. This could have been caused by insect pest, as aphids where observed in cucumber fields where samples were collected. These findings equally agrees with (Coutts *et al*., 2011) who reported that ZYMV is transmitted non-persistently by a number of aphids.

Differences in the infection rate of CMV, WMV and ZYMV in the various LGAs were observed between the dry and rainy seasons. The occasional difference on the viral infection rate could be because of changing ecological conditions which changes the conduct of the host plants, the biology of the vectors, transmission rate of the infection and viral replication rates (Hull, 2009). It could also be due to intrinsic factors of the crops including susceptibility and agricultural practices employed within the survey areas. Mixed virus infection in plant can fuel the severity of diseases and their symptoms, which in turn leads to a significant loss in yield (Malik *et al*., 2010). In the present study, mixed infections in Cucumber crops by CMV, WMV and ZYMV were detected in Sabon Gari, Kudan, Minjibir and Bunkure LGAs. Similar results were observed by Barbosa *et al*., (2016), based on molecular analysis of cucurbit samples collected from San Francisco valley in Brazil. It has been reported that mixed infections in cucurbits are very often detected in natural conditions between viruses from Potyvirus genus and CMV (Barbosa *et al*., 2016). Syller (2012) reported that multiple infections lead to a diversity of intra host virus–virus

interactions, many of which may result in the generation of variants showing novel genetic features, and thus change the genetic structure of the viral population.

5.2 Identification of Alternative Weed Hosts for *Cucumber Mosaic Virus, Watermelon Mosaic Virus* and *Zucchini Yellow Mosaic Virus*

Weeds assume a significant part as foci of infection from which there is spread into or within crop fields (Thresh, 1982). Consequently, the detection of CMV, WMV and ZYMV in the weed samples collected from the cucumber fields surveyed. This may partly account for the incidence of virus disease in the various LGAs. CMV was found to be the most infectious virus, as it was detected in *Portulaca oleracea* Linnaeus (family *Portulaceae*), *Euphorbia hirta* Linnaeus (family *Euphorbiaceae*), *Commelina coelestis* Willd (family *Commelinaceae*), *Chenopodium album* Aellen (family *Amaranthaceae*), *Mitacarpus hirtus* Linnaeus (family *Rubiaceae*), and *Ageratum houstonianum* Linnaeus (family *Asteraceae*). The high incidence of CMV in the weed samples is not new. A relative study carried out in Southwest Nigeria indicated 78.6% incidence of CMV in *Euphorbia hirta* Linnaeus (family *Euphorbiaceae*) and *Synedrella nodiflora* Gaertn (family *Asteraceae*) (Ayo-John *et al*., 2014). This finding therefore agrees with the report of van Regenmortel *et al*. (2000) that *Bromoviridae* including CMV, are one of the most important widespread viruses in the world with a very wide host range. CMV is seed-borne having a transmission rate between 1 and 50% (Palukaitis and García-Arenal, 2003), occurs in high concentration and is highly stable, hence its high incidence in the weeds as well as in Cucumber plants sampled in this study.

Watermelon mosaic virus was detected in *Euphorbia indica* Boiss (family *Euphorbiaceae*), *Alternanthera brasiliana* Kuntze and *Chenopodium murale* of the family *Amaranthaceae*, and *Waltheria indica* (family *Fabaceae*). Lecoq *et al*., (2014) reported that ZYMV commonly

occurs in cucurbit crops in many world region, but naturally occurring alternative hosts have proven difficult to find as their occurrence is sporadic and incidence very low, Coutts *et al* (2011) also reported that although ZYMV epidemics are common in many cucumber growing areas of the world it has a restricted host range. Their occurrence was also reported as often sporadic, incidence low even when inoculum pressure is very high, this could be why none of the weed samples sampled in this study identified any of them as alternative host of ZYMV.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 Summary

Field surveys were conducted on cucumber farms in 2020 dry and rainy season to serologically detect the incidence and distribution of the viruses (*Cucumber mosaic virus, Watermelon mosaic virus and Zucchini yellow mosaic virus*), and also to find their alternative hosts. A total of thirty-six farms were surveyed in Kaduna and Kano states, eighteen in each State, a total of 540 cucumber leaf samples and 180 weeds leaf samples were collected during dry and rainy season from the two states. The presence of these viruses were detected using the Double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA). The virus incidence and distribution in cucumber and weed hosts were determined in each LGA of the states. These results confirmed the presence of all of the three viruses (CMV, WMV and ZYMV) in both single and mixed infection in all of LGAs.

Weed hosts found to be serving as reservoir for these viruses include: *Portulaca oleracea, Euphorbia indica, Waltheria indica, Alternanthera brasiliana, Chenopodium album, Chenopodium murale, Mitracarpus hitrus, Epilobium hirsutum, Arthraxon hispidus, Ageratum houstonianum* and *Synedrella nodiflora.*

6.2 Conclusion

1. This study has for the first time established the incidence of these three viruses in Kaduna and Kano States during the dry and rainy seasons as follows:
    - In Kaduna State: In the dry season CMV (10%), WMV (3.67%) and ZYMV (0.73%), CMV+WMV (4.45%) and CMV+ZYMV (2.22%)
    - While during the rainy season CMV (16.30%), WMV (4.44%), ZYMV (5.18%)

- In Kano State: In the dry season CMV (5.18%), WMV (11.85%), ZYMV (0.74%), CMV+WMV (4.44%) and CMV+ZYMV (2.33%)
- During the rainy season CMV (18.53%), WMV (1.48%) and ZYMV (4.45%).

2. This is the first report of ZYMV, mixed infection of CMV+ZYMV and mixed infection CMV+WMV on Cucumber in Nigeria.

3. Weed host of CMV were: *Portulaca oleracea, Chenopodium album, Ageratum houstonianum* while the alternative host of WMV was found to be *Alternanthera brasiliana*.

4. This is the first report of *Ageratum houstonianum* as alternative host of CMV and *Alternanthera brasiliana* as alternative host of WMV in Nigeria and *Synedrella nodiflora* to harbour ZYMV.

6.3 Recommendations

1. Further studies on *Cucumber mosaic virus, Watermelon mosaic virus* and *Zucchini yellow mosaic virus*, should be conducted in other agro-ecological zones to establish its incidence and distribution in other agro-ecological zones.
2. ZYMV may be a recent introduction to the country, therefore continuous surveillance should be done to prevent it from spreading further since it has been reported to cause up to 100% yield loss in endemic areas.
3. Molecular tools which are more sensitive such as High-throughput Sequencing (HTS), Polymerase chain reaction (PCR) should be employed to determine the occurrence of the viruses considering the limitations of ELISA.

4. Farmers should use virus free seeds/ certified seeds/ seeds from certified sources and employ good weed management practices, as weeds can serve as reservoirs from where viruses can be spread.

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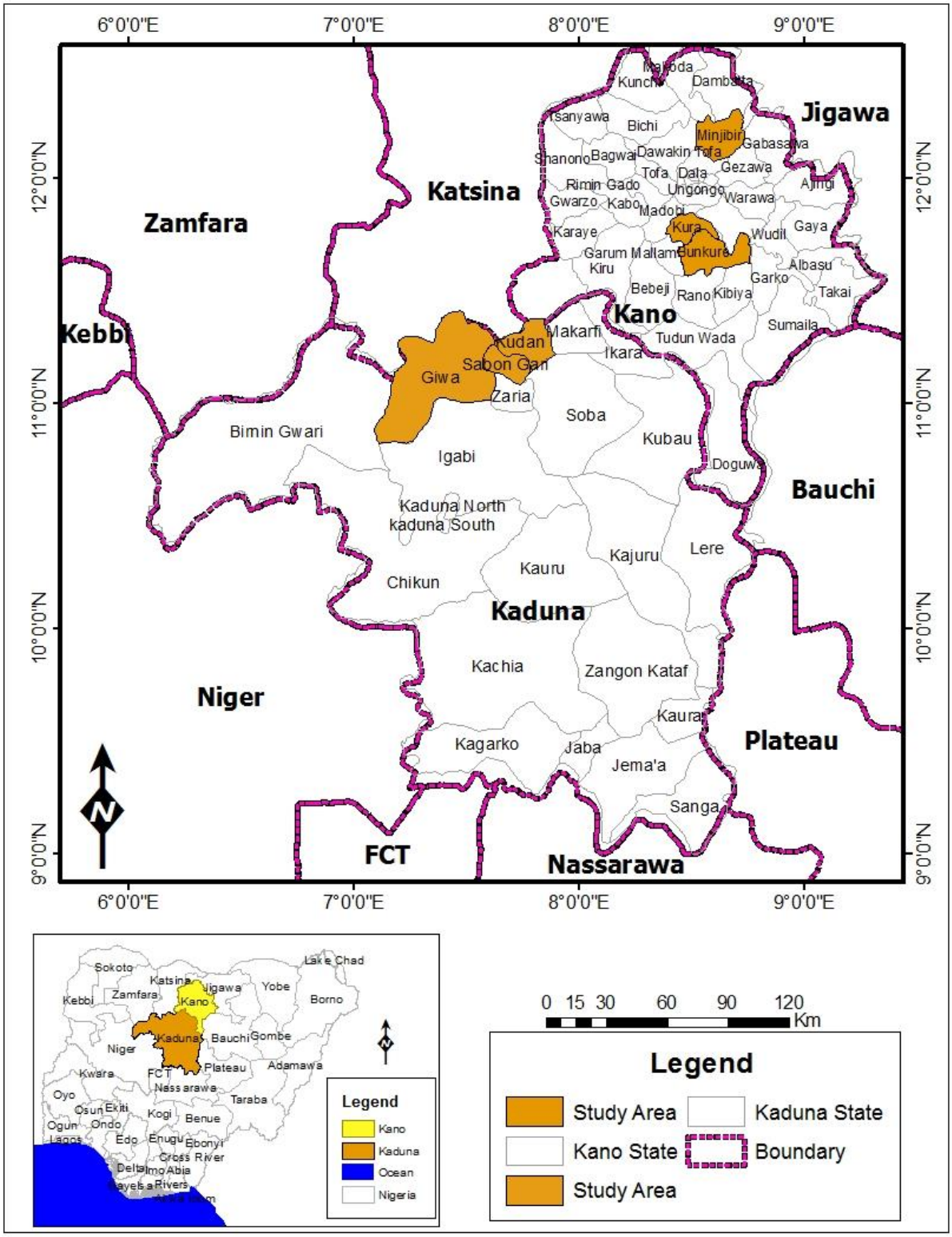
APPENDICES

Appendix I: Map showing the local government areas surveyed in Kaduna and Kano States



Appendix II: Questionnaire

1. Farm: ……..... GPS: …….
2. Village: …… L.G.A. ……. State……...
3. Farm Size …….
4. Variety …….
5. Cropping History …….
6. Source (s) of Seed ……
7. Planting Date ……
8. Plant Age (weeks) ……
9. Fertilizer Application ……
10. Cropping pattern ……
11. Weed control (Manual and/ Herbicides) ……
12. Disease/ Insect pests Observed (if any) …….
13. Pest Control Method (s) …….
14. Pesticides Used ……
15. Symptoms ……
16. Comments ……

Appendix III: Buffers Prepared for Use in 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)	800 ml distilled water was dissolved in and adjusted to pH 9.8 with HCl and filled to one litre

Appendix IV: Cropping Information of the Locations Surveyed in Kaduna and Kano State during the 2020 dry season (Feb-Apr)

LGA	LOCATION	No. of plants tested/field	Farm size (ha)	Insects Observed	Sanitary condition	Surrounding crops	Age of Plant	Cropping pattern
	Dan Mhawiya	15	0.72	Beetle, aphid	Weedy	Cabbage,Onion	6weeks	Cucumber with Maize and Tomatoes
	Giwa Sabuwa	15	0.56	Grasshoppers, Aphid	Weedy	Maize	4weeks	Cucumber with Maize
Giwa	Rafin	15	0.67	Grasshopper, Aphid, Ant	Weedy	Maize	7weeks	Cucumber with Pepper
	Hunkuyi	15	0.019	Grasshoppers, Aphid	Weedy	Cabbage,	7weeks	Cucumber with sweet potato, tomatoes
	Mai gaya musawa	15	0.33	Ants, Whitefly, Aphid	Weeded	Watermelon, Onion	6weeks	Cucumber with Guava, sweet melon, w/melon
Kudan	Jaja	15	0.124	Grasshopper, Aphid	Weeded	Maize, grape	8weeks	Cucumber Sole cropped
	Bomo	15	0.018	Black ants, Aphids, whitefly	Weedy	Maize, pepper	4 weeks	Cucumber with Onion, tomato, Maize
	Hayin Mallam	15	0.62	Aphids, Grasshoppers, whitefly	Weedy	Maize, Sweet melon	6weeks	Cucumber with Maize, peas, tomatoes
Sabon Gari	Shika	15	0.2	Aphids, Beetle, whitefly	Weedy	Cucumber, Tomatoes	4weeks	Cucumber Sole cropped
	Gaba da Gari	15	0.204	Grasshoppers	weeded	Tomatoes, Cucumber	6weeks	Cucumber mixed with Tomatoes
	Zango	15	0.74	Black ants, Aphids	weedy	Sorghum, Tomatoes, Pepper	6weeks	Cucumber mixed with Maize
Bunkure	Bunkure Gabas	15	0.075	Aphids	Weeded	Cucumber, Tomatoes	7weeks	Cucumber with Tomatoes and Cowpea

	Kadani	15	0.203	Aphids,Blackants, whitefly	Weedy	Cucumber, Pumpkin	8weeks	Cucumber sole cropped
	Dauni	15	0.283	Grasshoppers, aphids	weeded	Watermelon, Maize, Onion	7weeks	Cucumber with Watermelon and Maize
Kura	Gamadam	15	0.278	Ants, Beetle, Aphid	Weeded	Onion, Pepper, Maize	5weeks	Cucumber mixed with Maize
	Farin Gada	15	0.424	Aphids, Beetles, whitefly	Weedy	Watermelon, Onion	4weeks	Cucumber Sole cropped
	Wasai	15	0.32	Grasshoppers,Beetles	Weedy	Onions, Pepper	5weeks	Cucumber Sole cropped
Minjibir	Marke	15	0.333	Aphids, Whitefly, Black ants	Weedy	Watermelon, Onion	4weeks	Cucumber Intercropped with Maize

Appendix V: Cropping Information of the Locations Surveyed in Kaduna and Kano State during the 2020 rainy season (Aug-Oct)

LGA	LOCATION	No. of plants tested/field	Farm size (ha)	Insects Observed	Sanitary condition	Surrounding crops	Age of Plant	Cropping pattern
Giwa	Panhauya	15	0.236	Ants, grasshoper	Weeded	Tomatoes, Maize, Sorghum	7weeks	Cucumber with Pepper, Okra, Cabbage, Tomatoes, Carrot
	Abadawa	15	0.32	Ants, beetles, Aphids	Weeded	Maize, Cassava,Pepper	8weeks	Cucumber Sole cropped
	Angwan Makama	15	0.253	Whitefly, Red ant, black ant	Weeded	Pepper, Sorghum	9weeks	Cucumber with Watermelon, maize
Kudan	Jaja	15	0.411	Red ants. Black ant, Aphids	Weeded	Guava, Grape, Egg plant	6weeks	Cucumber with Sugarcane, Pepper, Cabbage
	Mai gaya Musawa	15	0.346	Beetle, whitefly, G/hopper	Weeded	Onion, Carrot, Cabbage	7weeks	Cucumber with Garden Egg, Sorghum, Maize
	Hunkuyi	15	0.212	Black ants, Grasshoppers	Weedy	Maize, Sorgum, S/Cane	8weeks	Cucumber with Groundnut, rice, Zobo
Sabon Gari	Bomo	15	0.324	Grasshoppers, Aphids	Weedy	Watermelon, Cabbage	7weeks	Cucumber mixed Maize, Tomato, Cassava
	Hayin Mai dakali	15	0.234	Beetle, Aphid, Ants	Weedy	Maize, Pepper, Onion	7weeks	Cucumber, Garden Egg
	Angwan Bisa	15	0.485	Aphids, Beetles, Whitefly	Weedy	Millet, Cassava	8weeks	Cucumber intercropped with Cabbage, watermelon
Bunkure	B/Gabas	15	0.4	Beetle, whitefly, aphids	Weeded	Maize, Cabbage, Lettuce	4weeks	Cucumber Sole cropped
	Gaba da gari	15	0.401	Red ants, Aphids	Weedy	Cucumber, Onion	6weeks	Cucumber Sole cropped
	Zango	15	0.305	Beetle, whitefly, black ants	Weeded	Cassava, Sorgum, S/cane	6weeks	Cucumber Sole cropped
Kura	Dauni	15	0.233	Whitefly, Aphids, Red ants	Weeded	Cassava, Carrot, Tomatoes	5weeks	Cucumber with Pumpkin, Mazie

	Kadani	15	0.311	Beetle, Aphid, Ants	Weeded	Sorghum, Cucumber, Maize	5weeks	Cucumber Sole cropped
	Gamadam	15	0.207	Black ants, Aphids	Weedy	Watermelon, pepper	7weeks	Cucumber intercropped with Cabbage, Pumpkin,
Minjibir	Farin Gada	15	0.307	Grasshoppers, Aphids	Weeded	Cabbage, Peas	5weeks	Cucumber Sole cropped
	Wasai	15	0.248	Whitefly, beetles	Weeded	Maize	4weeks	Cucumber with Pepper, Watermelon
	Marke	15	0.250	Aphids, Ants,	Weedy	Watermelon, Pepper	4weeks	Cucumber with Maize

Appendix VI: Elisa CMV reading for Kaduna and Kano States during the 2020 dry season

0.06	0.052	0.062	0.057	0.058	0.056	0.065	0.05	0.063	0.062	0.066	0.058
0.056	0.292	0.287	0.323	0.275	0.34	0.267	0.309	0.272	0.302	0.274	0.071
0.056	0.248	0.226	0.256	0.27	0.362	0.235	0.192	0.286	0.21	0.308	0.082
0.062	0.234	0.242	0.267	0.267	0.447	0.191	0.304	0.28	0.354	0.247	0.077
0.051	0.218	0.249	0.21	0.192	0.241	0.246	0.182	0.206	0.204	0.241	0.081
0.047	0.56	0.36	0.298	0.265	0.274	0.305	0.29	0.252	0.289	0.245	0.07
0.05	0.27	0.281	0.269	0.255	0.304	0.214	0.19	0.242	0.273	0.254	0.059
0.063	0.055	0.047	0.051	0.061	0.058	0.061	0.054	0.049	0.051	0.053	0.055

0.123	0.068	0.043	0.049	0.055	0.047	0.05	0.048	0.051	0.096	0.066	0.088
0.135	0.367	0.213	0.245	0.369	0.239	0.252	0.325	0.417	0.38	0.275	0.077
0.073	0.254	0.207	0.243	0.251	0.228	0.292	0.289	0.272	0.292	0.278	0.081
0.066	0.207	0.225	0.196	0.257	0.399	0.191	0.28	0.265	0.269	0.288	0.08
0.046	0.235	0.226	0.28	0.223	0.26	0.274	0.26	0.277	0.262	0.295	0.075
0.063	0.241	0.227	0.283	0.228	0.243	0.238	0.234	0.207	0.313	0.244	0.069
0.066	0.238	0.226	0.283	0.211	0.168	0.184	0.236	0.2	0.215	0.211	0.063
0.058	0.048	0.036	0.053	0.038	0.048	0.046	0.047	0.046	0.048	0.05	0.053

Appendix VII: Elisa WMV reading for Kaduna and Kano States during the 2020 dry season

0.088	0.082	0.061	0.046	0.047	0.063	0.05	0.049	0.059	0.055	0.055	0.058
0.053	0.164	0.125	0.135	0.136	0.121	0.162	0.136	0.137	0.167	0.178	0.067
0.066	0.128	0.128	0.138	0.123	0.122	0.156	0.134	0.128	0.13	0.142	0.071
0.057	0.164	0.115	0.136	0.119	0.307	0.152	0.139	0.137	0.14	0.155	0.073
0.053	0.193	0.113	0.123	0.128	0.114	0.118	0.13	0.121	0.139	0.12	0.068
0.058	0.153	0.107	0.102	0.117	0.114	0.119	0.153	0.112	0.114	0.12	0.065
0.062	0.25	0.146	0.101	0.11	0.099	0.107	0.128	0.133	0.13	0.121	0.051
0.055	0.052	0.053	0.058	0.056	0.054	0.059	0.053	0.058	0.053	0.054	0.06

0.073	0.058	0.043	0.053	0.045	0.049	0.067	0.049	0.042	0.085	0.066	0.059
0.07	0.329	0.142	0.121	0.143	0.137	0.152	0.158	0.138	0.151	0.182	0.055
0.052	0.139	0.116	0.113	0.123	0.129	0.125	0.127	0.187	0.124	0.143	0.059
0.054	0.213	0.129	0.115	0.126	0.403	0.131	0.148	0.128	0.124	0.142	0.073
0.053	0.116	0.114	0.11	0.115	0.121	0.119	0.124	0.113	0.123	0.13	0.054
0.057	0.127	0.112	0.115	0.047	0.107	0.099	0.097	0.103	0.121	0.11	0.075
0.05	0.124	0.098	0.094	0.179	0.109	0.112	0.095	0.109	0.111	0.105	0.051
0.053	0.048	0.052	0.051	0.049	0.044	0.06	0.054	0.047	0.045	0.054	0.06

Appendix VIII: Elisa ZYMV reading for Kaduna and Kano States during the 2020 dry season

0.064	0.062	0.044	0.052	0.054	0.069	0.057	0.062	0.057	0.056	0.056	0.054
0.052	0.173	0.165	0.142	0.147	0.141	0.285	0.151	0.16	0.174	0.169	0.07
0.059	0.136	0.151	0.14	0.135	0.152	0.147	0.159	0.168	0.159	0.163	0.067
0.055	0.161	0.154	0.15	0.15	0.819	0.144	0.16	0.22	0.159	0.183	0.072
0.052	0.14	0.143	0.139	0.144	0.14	0.122	0.134	0.15	0.13	0.148	0.07
0.055	0.15	0.126	0.142	0.167	0.13	0.134	0.141	0.151	0.135	0.129	0.063
0.057	0.201	0.15	0.141	0.158	0.135	0.144	0.118	0.138	0.131	0.129	0.055
0.089	0.067	0.043	0.054	0.07	0.057	0.064	0.055	0.054	0.052	0.055	0.06

0.057	0.063	0.059	0.055	0.172	0.058	0.06	0.05	0.065	0.074	0.075	0.099
0.056	0.144	0.129	0.123	0.154	0.145	0.155	0.152	0.153	0.166	0.211	0.067
0.053	0.142	0.13	0.149	0.143	0.145	0.143	0.186	0.147	0.146	0.176	0.071
0.047	0.143	0.154	0.15	0.132	0.636	0.148	0.151	0.158	0.155	0.164	0.067
0.05	0.138	0.15	0.134	0.15	0.177	0.154	0.166	0.185	0.16	0.187	0.073
0.049	0.159	0.147	0.133	0.135	0.147	0.145	0.143	0.155	0.153	0.196	0.055
0.055	0.22	0.222	0.134	0.123	0.133	0.139	0.146	0.153	0.163	0.198	0.064
0.066	0.055	0.055	0.053	0.068	0.065	0.063	0.054	0.057	0.068	0.05	0.056

Appendix IX: Elisa CMV reading for Kaduna and Kano States during the 2020 rainy season

0.047	0.046	0.096	0.049	0.05	0.088	0.054	0.05	0.067	0.067	0.059
0.043	0.316	0.236	0.314	0.478	0.597	0.402	0.434	0.482	0.394	0.327
0.055	0.537	0.259	0.378	0.308	0.444	0.528	0.411	0.548	0.467	0.535
0.039	0.356	0.466	0.399	0.243	0.492	0.228	0.677	0.764	0.64	0.546
0.039	0.397	0.343	0.323	0.35	0.472	0.305	0.372	0.325	0.504	0.461
0.041	0.271	0.317	0.315	0.287	0.204	0.637	0.424	0.363	0.272	0.363
0.049	0.425	0.241	0.395	0.452	0.426	0.058	0.07	0.044	0.045	0.043
0.07	0.054	0.06	0.071	0.092	0.043	0.051	0.054	0.049	0.052	0.054

0.056	0.052	0.079	0.049	0.069	0.05	0.046	0.086	0.049	0.057	0.057	0.058
0.052	0.223	0.18	0.238	0.244	0.169	0.274	0.251	0.259	0.219	0.323	0.081
0.052	0.157	0.189	0.162	0.15	0.164	0.266	0.213	0.22	0.242	0.211	0.108
0.06	0.174	0.155	0.179	0.173	0.304	0.198	0.219	0.218	0.291	0.233	0.111
0.048	0.189	0.17	0.144	0.152	0.14	0.161	0.166	0.178	0.264	0.215	0.091
0.043	0.18	0.165	0.158	0.169	0.216	0.267	0.222	0.186	0.219	0.269	0.068
0.049	0.197	0.18	0.185	0.638	0.527	0.049	0.05	0.048	0.051	0.055	0.069
0.058	0.052	0.052	0.05	0.052	0.06	0.05	0.046	0.051	0.055	0.047	0.048

Appendix X: Elisa WMV reading for Kaduna and Kano States during the 2020 rainy season

0.067	0.054	0.083	0.073	0.126	0.109	0.116	0.155	0.064	0.076	0.095
0.056	0.231	0.173	0.162	0.147	0.237	0.248	0.163	0.14	0.169	0.297
0.055	0.161	0.149	0.173	0.209	0.209	0.161	0.23	0.17	0.276	0.212
0.05	0.146	0.128	0.224	0.168	0.357	0.161	0.222	0.205	0.182	0.145
0.058	0.14	0.158	0.195	0.361	0.24	0.134	0.151	0.175	0.138	0.181
0.047	0.157	0.233	0.216	0.223	0.159	0.231	0.223	0.187	0.16	0.179
0.047	0.193	0.244	0.146	0.186	0.242	0.049	0.047	0.041	0.05	0.047
0.054	0.054	0.057	0.052	0.054	0.048	0.051	0.051	0.049	0.051	0.052

0.054	0.062	0.048	0.074	0.056	0.156	0.06	0.066	0.046	0.079	0.051
0.073	0.866	0.514	0.322	0.249	0.408	0.276	0.193	0.208	0.33	0.658
0.061	0.258	0.17	0.188	0.176	0.172	0.175	0.242	0.197	0.23	0.461
0.063	0.476	0.397	0.328	0.268	0.42	0.285	0.278	0.279	0.274	0.337
0.042	0.251	0.22	0.155	0.164	0.175	0.23	0.248	0.224	0.196	0.215
0.045	0.187	0.178	0.23	0.213	0.208	0.243	0.234	0.23	0.223	0.252
0.059	0.316	0.204	0.204	0.218	0.206	0.054	0.052	0.046	0.047	0.05
0.055	0.052	0.051	0.049	0.057	0.047	0.061	0.047	0.051	0.051	0.049

Appendix XI: Elisa ZYMV reading for Kaduna and Kano States during the 2020 rainy season

0.075	0.059	0.066	0.058	0.06	0.08	0.047	0.072	0.067	0.05	0.052	0.048
0.059	0.315	0.213	0.249	0.255	0.274	0.226	0.167	0.199	0.287	0.308	0.094
0.041	0.264	0.182	0.207	0.324	0.215	0.264	0.192	0.187	0.241	0.289	0.079
0.039	0.294	0.224	0.247	0.292	(+)	0.204	0.213	0.304	0.266	0.342	0.08
0.045	0.318	0.212	0.211	0.239	0.215	0.217	0.2	0.241	0.291	0.25	0.068
0.041	0.262	0.234	0.233	0.319	0.208	0.269	0.212	0.258	0.459	0.283	0.067
0.099	0.304	0.209	0.165	0.248	0.509	0.051	0.068	0.055	0.047	0.068	0.058
0.051	0.047	0.045	0.042	0.042	0.052	0.048	0.059	0.055	0.054	0.09	0.059

0.073	0.04	0.062	0.057	0.076	0.128	0.059	0.088	0.058	0.063	0.063	0.061
0.051	0.47	0.179	0.263	0.37	0.388	0.21	0.229	0.187	0.222	0.301	0.054
0.048	0.141	0.193	0.152	0.149	0.231	0.37	0.185	0.319	0.378	0.189	0.062
0.046	0.127	0.165	0.191	0.184	(+)	0.215	0.242	0.321	0.288	0.188	0.057
0.106	0.209	0.16	0.324	0.501	0.157	0.213	0.168	0.153	0.187	0.228	0.054
0.048	0.155	0.139	0.158	0.135	0.144	0.179	0.298	0.295	0.266	0.248	0.055
0.049	0.63	0.297	0.276	0.293	0.202	0.048	0.046	0.045	0.047	0.066	0.051
0.057	0.058	0.086	0.075	0.051	0.053	0.053	0.052	0.05	0.054	0.05	0.051

Appendix XII: Kaduna State Weather Report during the study months for 2020

Month	Humidity (%)	Min Temperature (^{0}C)	Max Temperature (^{0}C)	Rainfall (mm)
February	28.77	16.03	30.64	0.00
March	35.00	20.37	36.92	0.00
April	59.21	24.02	38.20	0.00
August	98.66	19.66	37.33	172.40
September	99.78	20.12	29.61	254.20
October	92.03	17.52	32.02	88.40

Appendix XIII: Kaduna State Weather Report during the study months for 2020

Month	Humidity (%)	Min Temperature (^{0}C)	Max Temperature (^{0}C)	Rainfall (mm)
February	9.89	15.29	31.47	0.00
March	9.23	19.73	38.87	0.00
April	8.53	26.09	41.96	0.00
August	35.79	22.00	29.74	17.60
September	29.92	22.41	31.91	3.40
October	12.78	20.74	34.66	3.50