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**Spatial distribution of virus diseases on okra (*Abelmoschus esculentus* L. Moench) plants and resistance to *Cucumber mosaic virus* disease in Minna, Nigeria**

## ABSTRACT

Okra is an important vegetable crop in the diet of millions of peoples in the tropical and subtropical regions of the world. Unfortunately, its yield is seriously threatened by several virus diseases, including *Cucumber mosaic virus* (CMV) and *Okra mosaic virus* (OkMV). To date, adoption of resistant cultivars is by far the most effective and sustainable management measure against plant pathogenic viruses. This study was conducted to determine the: (1) occurrence and distribution of CMV and OkMV diseases in selected Local Government Areas (LGAs) of Niger State, and (2) growth and yield responses of four genotypes of okra virus under CMV infection. A survey was carried out in six LGAs (Borgu, Bosso, Gbako, Mashegu, Mokwa and Paikoro) of Niger State in 2019 cropping season. Four communities were surveyed and five symptomatic leaf samples of crops were collected from each community amounting to 20 leaf samples from each Local Government Areas. A total of 120 symptomatic leaf samples were indexed for all the local government areas surveyed. The samples were subjected to Double Antibody Sandwich Enzyme-Linked Immunosaobent Assay (DAS-ELISA). The polyclonal antibodies (PAbs) used were *Cucumber mosaic virus* (CMV) and *Okra mosaic virus*(OkMV).The screenhouse evaluation of four okra genotypes was laid out in completely randomised design with three replicates. Okra seedlings were inoculated with CMV at 10 days after sowing (DAS) by sap transmission. Each genotype was evaluated as CMV-infected and control. Data on disease incidence and severity, growth and yield variables were subjected to analysis of variance (ANOVA) and means separated using the Least Significant Difference (LSD) at $p$=0.05 probability level. *Cucumber mosaic virus* and OkMV were detected during the survey, with the former being more prevalent. A total of 11 samples (9.2 %) reacted positively with CMV PAb. Incidence of CMV disease was highest in Borgu (3.3 %) and Mokwa LGAs (3.3 %), followed by Paikoro LGA (1.7 %) while an incidence of 0.8 % was found in Gbako LGA. On the other hand, none of the samples from Bosso and Mashegu reacted positively with CMV PAb. In Borgu LGA, CMV disease was found at Dogongari, Gadaoli, Fakun and Malele. In Gbako LGA, the virus was detected only at Emisomma community. In Mokwa LGA, the disease occurred at Government Teachers College, Waabi, Wuya-Kede and Rail Station. The two CMV-positive samples in Paikoro LGA came from Jankpan and Jyipe communities. On the other hand, only 2 samples (1.7 %) tested positive for OkMV disease. These were found at Gidan-Mugoro and Edokota, in Bosso and Gbako

LGA, respectively. Overall, "OKR_19_01" genotype exhibited the highest fruit length (5.3cm), fruit weight (4.3 g/plant) and fruit diameter (2.5 cm) under CMV infection. Therefore, cultivation of "OKR_19_01" okra genotype is recommended in CMV-endemic areas. Although100 % incidence of CMV disease was found on "OKR_19_01" plants as early as 1 WAI, it eventually gave the greatest yield performed among the okra genotypes evaluated. It is therefore, recommended to farmers in CMV-endemic locations for better productivity. However, further extensive survey is needed so as to reveal virus diversity in okra farmlands. In the meantime, the okra genotypes "OKR_19_01" could be adopted in CMV-endemic areas. Plant breeders could also cross this genotype with CMV-resistant genotype for improved resistance and yield.

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# CHAPTER ONE

## 1.0 INTRODUCTION

### 1.1 Background of the Study

Okra {*Abelmoschus esculentus* (L.) Moench} belongs to the kingdom *Plantae,* division *Magnoliophyta,* class *Magnoliopsida,* order *Malvales,* family *Malvalceae,* and genus *Abelmoschus*(Kumar *et al.,* 2010). It is commonly known as *Bhindi* in India, *Krajab Theaw* in Thailand, *Kopi* Arab, *Kacang bendi* and *Blinde* in South East Asia. In the Middle East it is known as *Bania, Banya* or *Banich* and *Gumbo* in southern USA, and lady's finger in England (Saifullah and Rabbani 2009). In Nigeria for instance it is known as *Ila* in Yoruba, *Okwuru* in Igbo and *Kubewa* in Hausa (Haruna and Jabil, 2017). It is also known as *Kpammi* in Nupe and *Kpani* in Gbagi speaking parts of Niger State. Formerly, okra plant was included in the genus *Hibiscus* and was later designated to *Abelmoschus* which is distinguished from the genus Hibiscus (Kumar *et al.,* 2010).

It is widely distributed in the tropics, sub tropics and warmer portions of the temperate region (Aladele *et al.,* 2008). Okra is mainly propagated by seed and has duration of 90-100 days (Kumar *et al.,* 2010). According to Aladele *et al.* (2008), it can be found in almost every market all over Africa. Most okra can be eaten when cooked or in processed form and the young fruits may be eaten raw. It is also reported that oil in the seeds could be higher than that of poultry egg and soyabean (Iyagba *et al.,* 2013). In Nigeria, the limiting factors in okra production and other vegetables among others include weed management, unproductive soils, tillage practices, low yielding varieties and sub optimal plant density (Iyagba *et al.,* 2013). Okra needs temperature above 20°C for normal growth and development (Abdel-kadel *et al.,* 2010). Germination percentage and speed of emergence

are optimal at 30-35°C (Abdel-kadel *et al.,* 2010). Accordingto Dada and Fayinminnu (2010), an average temperature of 20-30 °C is considered optimum for growing, flowering and fruiting. *Abelmoschus* species is a short – day plant, but its wide geographical distribution of up to latitudes of 35-40° indicates that cultivars differ markedly in sensitivity (Abdel-kadel *et al.,* 2010). Flower initiation and flowering are hardly affected by day length in popular sub-tropical cultivars such as Clemson spineless. Most tropical cultivars show quantitative short-day responses but also qualitative responses do occur (Abdel-kadel *et al.,* 2010). The shortest critical day length is 12 hours 30 minutes; this explains why flowering of local cultivars of common okra is only quantitatively affected by day length in coastal areas of the Gulf of Guinea (5°N). However, more inland at higher latitudes (10°N) one can occasionally observe very tall non-flowering plants of common okra due to a qualitative response. Common okra tolerates poor soil, but prefers well-drained sandy loams with pH 6-7 and high organic matter content (Adilakshmi *et al.,* 2010).

Okra is susceptible to various insect pests and diseases which may reduce yield optimization if not properly managed. Among the insect pests are the silver leaf, white fly, rough ball worm and green vegetable bugs (Fajinmi and Fajinmi, 2010). The occurrence of aphids and mites may also be noticed (Fajinmi and Fajinmi, 2010). The pathogens of okra include fungi, nematodes, bacteria and viruses (Fajinmi and Fajinmi, 2010). Frequent use of pesticides by farmers, without recognizing the vectors, its incidence patterns and the virus infection time, create poisonous residues in the food chain (Iyagba *et al.*, 2013).

## 1.2 Statement of the Research Problem

Okra is an important vegetable crop in Africa. It is rich in protein, minerals, vitamins and roughages (Benchasri, 2012). However, its productivity is challenged by a number of constraints ranging from abiotic and biotic factors. Reduction in okra yield by biotic factors includes attack by insect pests and diseases. *Cucumber mosaic virus* (CMV) is one of the most economically important viruses of okra (Bushra *et al.,* 2012). The virus particles multiply in the infected plant cells and change the biochemical compounds of cells such as chlorophyll, organic carbon, nucleic acids and β carotene (Bushra *et al.,* 2012). Similarly, Bushra *et al.* (2012) reported a decrease in total chlorophyll by 65% followed by 82% reduction in photosynthesis rate in *Luffa aegyptica* and metabolic changes in vegetable leaves due to *Cucumber mosaic virus* infection. Virus infection alters the gross form, arrangement and appearance of cells by disturbing their internal organisation. Some anatomical changes also exhibited due to the virus infection in vines include disintegration of tissue, hypertrophy, compactness of tissue and decrease in number of chloroplasts in the cells (Bushra *et al.,* 2012).

Virus diseases are important constraints in the production of okra worldwide as they affect their growth and productivity (Asare *et al.,* 2014). Okra mosaic disease caused by *Okra mosaic virus* (OkMV) is of the genus *Tymovirus* and family *Tymoviridae* and is the most prevalent virus disease of okra in West Africa (Asare *et al.,* 2014). It is the most common virus disease of okra with disease incidence of up to 100% recorded in some okra fields (Asare *et al.,* 2014). Incidence of okra mosaic disease has been reported in other West African countries including Cote d`Ivoire, Ghana and Nigeria (Fajinmi and Fajinmi, 2010).

## 1.3 Justification of the Study

*Cucumber mosaic virus* (CMV) has a wide range of host and infects a great variety of vegetables, ornamentals and other plants because it has as many as 191 host species in 40 families (Zitter and Murphy, 2009). Virus diseases may be particularly problematic in okra as indeed in other horticultural crops as a result of inadequate management measures (Bushra *et al.,* 2012). The disease caused by CMV also presents a variety of global management problems in a wide range of agricultural crops. The high magnitude of risk posed by CMV is due to its broad host range and high number of arthropod vectors (Bushra *et al.,* 2012). The ability of the virus to cause high severe losses in crops makes it an agricultural disease of major importance (Zitter and Murphy, 2009). The virus causes severe damages from mild to severe symptoms. Symptoms of CMV vary from mosaic to yellow mottles, stunting and leaf curling (Zitter and Murphy, 2009).

Managing okra mosaic disease is therefore quite important in order to improve yields and production of okra (Fajinmi and Fajinmi, 2010). Most of the researches on management of virus vectors are oriented with chemical control. However, *Okra mosaic virus* (OkMV) is very difficult to manage with insecticides. Breeding and planting of resistant varieties as well as alteration in date of planting is the most effective way of managing virus diseases. It is quite important to effectively manage these virus diseases in okra in order to improve yields and fruit quality. Information on the incidence and severity of these diseases is an important prerequisite for the development of an appropriate and effective management strategy (Asare *et al.,* 2018). Furthermore, information on farmers' knowledge level of these virus diseases is important in the development of effective management strategies.

## 1.4 Aim and Objectives of the Study

The aim of this study was to identify okra genotype (s) that are resistant to okra viruses in the study area.

The objectives of the study were to determine the:

**i.** occurrence and distribution of *Cucumber mosaic virus* and *Okra mosaic virus* on okra (*A. esculentus*) in some selected Local Government Areas of Niger State.

**ii.** growth and yield responses of four genotypes of okra virus under CMV infection.

# CHAPTER TWO

# 2.0 LITERATURE REVIEW

## 2.1 Botany and Growth Habits of Okra

Okra belongs to the kingdom *Plantae,* division *Magnoliophyta,* class *Magnoliopsida* order *Malvales* family *Malvaceae,* genus *Abelmoschus* and species *A. esculentus* (Ariyo and Lapene, 2008). Latin binomial names for okra are *Abelmoschus esculentus* (Kumar *et al*., 2010). It is an upright annual herbaceous plant of about 1 to 2 metres (m) tall with a hibiscus like flower (Abdel-Kadel *et al.,* 2010). Okra has a deep tap root, semi woody stem and sometimes pigmented with a green or reddish tinge colour. It is variable in branching with many short branches that are attached to a thick woody stem (Fajinmi and Fajinmi, 2010). The stem attains a height of 1 m in dwarf varieties as compared to 2 – 2.5 m in most varieties. The woody stem bears leaves that are lobed and are generally hairy, some up to 5 cm in length. Leaves are chordate (heart shaped); simple, usually palmtely 3-7 lobed and veinus leaves are subtended by a pair of narrow stipules.

According to Ariyo and Lapene (2008), okra leaf is dark green in color and resembles a maple leaf. The flowers are borne vertically only on the orthotropic axis every two or three days. The flower is axillary and solitary, borne on a peduncle 2.0-2.5cm long. The flowers are large, 5 cm in diameter with five white to yellow petals with a red or purple spot at the base of each petal and flower. The fruit is an elongated, conical capsule and five cavities ovules. The fruit is long, ribbed, developing in the leaf axial and spineless. The fruit is normally yellowish green to green but sometime purple or white and the pods are the edible portion (Ariyo and Lapene, 2008).

## 2.2 Growth Requirements of Okra

*Abelmoschus esculentus* needs temperature of above 20°C for normal growth and development and rainfall of 1000 cm -1500 cm per annum (Abdel-Kadel *et al.,* 2010). It is sensitive to frost and extremely low temperatures. Germination percentage and speed of emergences are optimal at 30 – 35°C (Akande *et al.,* 2010). Flower initiation and flowering are delayed with increasing temperatures as there is a positive correlation between temperature and number of vegetative nodes.

According to Dada and Fayinminnu (2010), an average temperature of 20°C to 30°C is considered optimum for growing, flowering and fruiting. *Abelmoschus* spp is a short-day plant, but its wide geographical distribution of up to latitudes 35°N– 40°S indicates that cultivars differ markedly in sensitivity. Flower initiation and flowering are hardly affected by day length in popular subtropical cultivars such as 'OKR19_01'. Most tropical cultivars show quantitative short-day responses, but qualitative responses also occur (Akande *et al.,* 2010). The shortest critical day length reported is 12 hours 30 minutes. However, more inland at higher latitudes (10°N) one can occasionally observe very tall non-flowering plants of common okra due to a qualitative response. Common okra tolerates poor soils, but prefers well-drained sandy loams, with pH 6-7, and a high content of organic matter (Adilakshmi *et al.,* 2010).

## 2.3 Origin and Geographical Distribution of Okra

Okra originated somewhere around Ethiopia and was cultivated by the ancient Egyptians by the 12$^{th}$ century BC. Its cultivation spread throughout the Middle East and North Africa (Tripathi *et al*., 2011). Okra is grown in many parts of the world, especially in tropical and sub-tropical countries (Arapitsas 2008; Saifullah and Rabbani 2009). *Abelmoschus* species

occur in the world as *A. moschatus, A.manihot A. esculentus, A. tuberculatus, A. ficulneus, A. crintus, A. angulosus* and *A. caillei*. Its cultivation in West Africa cuts across Guinea, Nigeria, Cameroon, Gabon and the Democratic Republic of Congo in Central Africa and Uganda in East Africa (Abidi *et al.,* 2014). The three cultivated species which are sometimes found in a semi-wild state in clearings, along roads and near villages, occur at low altitudes in all tropical, subtropical and warm temperate regions of the world (Tripathi *et al*., 2011).

The species *A. moschatus* has a wide geographical distribution in India, Southern China, Indonesia, Papua New Guinea, Australia, Central and West Africa. The species *A. manihot subsp. Manihot* is cultivated mainly in East Asia, but also in the Indian sub-continent and Northern Australia (Tripathi *et al*., 2011). The species *A. manihot subsp. Tetraphyllus* comprises of two wild forms differentiated on the basis of their ecological adaptation. First *var-tetraphyllus*, grows at low altitudes between 0 and 400m in the regions with a marked dry season in Indonesia, Philippines, Papua New Guinea and New Ireland. Second, *var. punens*, grows at altitude between 400 and 1600m in the Philippine and Indonesia (Tripathi *et al*., 2011). The species *A. esculentus* is cultivated as a vegetable in most tropical and subtropical regions of Africa such as Ghana, Guinea, Ivory Coast, Liberia and Nigeria. Arapitsas (2008), stated that the wild species *A. ficulneus* is found in a vast geographic area stretching from Africa to Asia and Australia. It flourishes in tropical areas of low altitude with a long dry season. Okra plants are grown commercially in many places such as India, Japan, Turkey, Iran, West Africa, Yugoslavia, Bangladesh, Afghanistan, Pakistan, Myanmar, Malaysia, Thailand, Brazil, Ethiopia and Cyprus (Tripathi *et al*., 2011). The species *A. caillei* is a multipurpose crop and its existence points directly to West and

Central Africa. It has been reported from Guinea to Nigeria in West Africa and Cameroon, Gabon and Dr Congo in Central Africa (Mathew *et al.,* 2016).

## 2.4 Okra Production Statistics and Economic Importance

World production of okra fruits was estimated at 8 million tons per year, and in West Africa it was estimated at 2,484,701.00 tonnes per year (Food and Agriculture Organization, 2017). Estimated total harvested area of okra in West Africa is 1,545,684 ha and Nigeria occupying the largest total area of 1,463,436 ha compared with 13,115, 4,035, 3,160, 651 and 516 hectares by Benin Republic, Cote d'Ivoire, Ghana, Senegal and Kenya, respectively (FAO, 2017). Okra production constitutes about 4.6 percent of the total staple food production in Nigeria between 1970 –2003. The West and Central Africa regions account for more than 75% of okra produced in Africa, but the average productivity in the region is very low with 2.5 t/ha compared to East with 6.2 t/ha and North with 8.8 t/ha. Ngbede *et al.* (2014) also reported that okra is consumed throughout Nigeria. According to Kator *et al.*(2015), the average growth and rate of vegetable crop including okra produced in Nigeria between 1989 and 1993 was 14.0 % compared to 6.4 % of cassava, 18 % for palm oil and 3.8 % for maize. Nigeria is one of the largest producers of okra in Africa with 1,978,286 tonnes, followed by Cote d'Ivoire and Ghana (FAO, 2017). However, yield in Nigeria is put at 1.35 t/ha compared with 29.33, 21.00, 7.64, 3.22, and 2.75 t/ha in Senegal, Ghana, Kenya, Benin republic and Cote d'Ivoire respectively (FAO, 2017). Okra has huge potential to enhance livelihoods in urban and rural areas and several stakeholders (Kator *et al.,* 2015). It offers a possible route to prosperity for small and large producers alike and all those involved in the okra value chain, including women producers and traders (Kumar *et al.,* 2010).

**Table 2.1 Top ten okra producing countries in the world and Africa.**

| World | | | Africa | | |
|---|---|---|---|---|---|
| Country | Area harvested (ha) | Production (Tonnes) | Country | Area harvested(ha) | Production (Tonnes) |
| Albania | 1620 | 6176000 | Benin | 1802463 | 1819018 |
| Bahamas | 1620 | 1819018 | Burkina_Faso | 148431 | 512855 |
| Bahrain | 1620 | 512855 | Cabo Verde | 54061 | 309413 |
| Barbados | 1620 | 309413 | Chad | 46741 | 152325 |
| Belize | 1620 | 152325 | Congo | 36975 | 104216 |
| Benin | 1617 | 124779 | Djibouti | 29365 | 103854 |
| Brunei_Darussalam | 1614 | 104216 | Egypt | 16932 | 88819 |
| Burkina Faso | 1613 | 103854 | Ghana | 5719 | 68954 |
| Cameroon | 1612 | 88819 | Kenya | 3744 | 60341 |
| Congo | 1610 | 68954 | Malawi | 3270 | 35200 |

Source: Food and Agriculture Organization (2019)

## 2.5 Uses of Okra

The production of okra as vegetable in Nigeria has rapidly increased in recent years. The seasonal supply of this vegetable to a large extent determines how much of it is being consumed by the majority of the people. Okra is suitable for cultivation as a garden crop as well as on large commercial farms. Okra contains proteins, carbohydrate and vitamin C (Dilruba *et al.,* 2009). Okra plays a vital role in human diet and consumption of young immature okra pods is important as fresh fruit and it can be consumed in different forms. Fruits can be boiled, fried or cooked (Akintoye *et al.,* 2011). The composition of okra pods per 100g edible portion (18 % of the products as purchased, ends trimmed is: water 88.6 g, energy 144.00 kj (36kcal), protein 2.10 g, fat 0.20 g fibre 1.70 g ca 84.00 mg, p 90.00 mg, fe 1.20 mg riboflavin 0.08 mg, thiamin 0.04 mg, niacin 0.06 mg, ascorbic acid 4.7 (Varmudy, 2011). The fresh and green tender fruit of okra which is called mucilage is used as vegetable. Carbohydrates in okra are mainly present in the form of mucilage (Kumar *et al.,* 2009). The oil in the seed of okra could be as high as in poultry eggs and soyabean (Akintoye *et al.*, 2011). Okra seeds can be dried, and are nutritious materials that can be roasted and ground to be used as coffee additive or substitute (Akintoye *et al.,* 2011). Okra leaves are considered good cattle feed and okra mucilage is suitable for industrial and medical application (Varmudy, 2011). Industrially, okra mucilage is usually used for glace paper production and also has a confectionery use. Okra is medicinal as plasma replacement or blood volume expander (Kumar *et al*., 2009). Okra is said to be useful against genitor-urinary disorders, spematorrhoea and chronic dysentery (Kumar *et al*., 2009). Its medicinal value has also been reported in curing ulcers and relief from hernorrhoids (Kumar *et al.,* 2009). The immature seed pods are eaten as a vegetable in various countries and used to thicken soups and stews

while the roots and stems are used for preparing "guru" or the brown sugar. It is the mucilage that contains most of the good soluble fibre which helps lower serum cholesterol (Adetuyi *et al.,* 2008). Okra also contains insoluble fiber which helps improve the health of the intestinal tract and lower the risk of some cancer patients (Kumar *et al.,* 2009).

## 2.6 Virus Diseases of Okra

Okra is an important vegetable grown in Nigeria and it can be found in almost every market in West Africa (Aladele *et al.,* 2008). Despite its importance, okra is faced with so many virus diseases (Fajinmi and Fajinmi, 2010). The incidence pattern of insect pests and diseases are more or less common to all growing areas. *Yellow vein mosaic* (YVM) is one of the most pressing plant protection problem universally faced by all okra growers (Benchasri, 2012). This Jassid (*Amxasca bigottula*) transmitted virus disease seriously stunts plant growth, reduces available leaf area for photosynthesis resulting into serious yield and quality loss. The vein and veinlets of affected plants turn yellow and even the fruit produced by such plants turn yellow in colour. The viruleferous population of vectors has potential to turn a large tract of healthy okra crop into an unproductive one in a short span of time. Virus resistant and tolerant commercial varieties ($F_1$ hybrid and Open Pollination) are available for management of this disease in countries like India and Japan. In addition, insecticides are also used to control its vectors.

### 2.6.1 *Okra mosaic virus* (OkMV)

*Okra mosaic virus* belongs to the genus *Tymovirus* and was first found in *Hibiscus esculentus* in Cote d'ivoire (Alegbejo, 2015). It is unipertite and consists of 32 and 68 % nucleic acid

and protein respectively. The virions are found in the leaves and roots and the thermal inactivation point is 80° C, longevity *in vitro* is 7 -9 days, while the dilution endpoint is $10^{-6}$. The virus contains a single stranded positive-sense RNA and with isometric particle of 28nm in diameter (Fajinmi and Fajinmi, 2010). *Okra mosaic virus* was initially reported in Cote d'ivoire and Nigeria only (Alegbejo, 2015) but it is likely to be widespread in many West African countries.

#### 2.6.1.1 Host range

The natural hosts of OkMV include jute mallow (*Corchorus olitorius*), Roselle (*Hibiscus sabdariffa),* Hibiscus (*H. rosa-sinensis*) and West African okra (*Abelmoschus caillei*) (Asar*e et al.,* 2018). Other host plants include *Abelmoschus esculentus* (L.) Moench, *Adansonia* sp., *Amaranthus* sp. *Chenopodium album* L., *C. amaranticolor* Coste and Reyn, *Arachishy pogaea* L., *Cieba* sp., *Citrullus lanatus* (Thunberg), *Corchorus* spp., *Cucumis sativus* L., *Dianthus barbartus* L., L., *Gossypium hirsutum,. Hibiscus cannabinus* L., *Lavatera* spp., *Mathiola* spp., *Mamodica* spp., *Nicotiana benthamiana* Domin, *N. clevelandii* L., P. *vulgaris* L., *phlox* spp., *Pisium sativum* L., *Sesanum indicum* L., *Sipnases* sp., *Solanum melogena* L., *S, nigrum* L., *Spinaciao leracea* L., *Tetragonia expansa* Murr., *Theobroma caccao* L., *Trifolium incarnates* L., *T. pretense* L., *Tropaelum sp., Vivaciafaba* L., *V. unguiculata* (L.) Walp. and *Voandezeia subterrenea* L. Thou. (Alegbejo, 2015).

#### 2.6.1.2 Transmission

This virus is transmitted in a non-persistent manner by the Coleopteran (Flee beetle) *Podagrica decolorata* (Alebgejo, 2015). It is also sap-transmissible. *Okra mosaic virus* is

very difficult to control with insecticide or by eliminating the virus hosts. There is scarcity of information on host resistance to OkMV. New okra crops should not be planted close to the infected crops. *Okra mosaic virus* has always been a serious problem in okra (Fajinmi and Fajinmi, 2010). Yield reductions of 20 – 50% have been reported and this loss may increase to 90% in susceptible varieties (Fajinmi and Fajinmi, 2010).

#### 2.6.1.3 Disease symptoms

*Okra mosaic virus* symptoms are characterised by a homogenous interwoven network of yellow mosaic pattern enclosing islands of green tissue in leaf blades. In extreme cases, infected leaves become yellowish or creamy in colour and the plant severely stunted (Alegbejo, 2015). Typical symptoms of OkMV disease include vein-chlorosis, vein-banding, mosaic, and stunting.

#### 2.6.1.4 Management of *Okra mosaic virus* disease

The integrated pest management constraints are that vectors usually attack the young okra plants at the vegetative stage for virus transmission. Frequent use of pesticides by the farmers, without recognising the vector(s), its incidence patterns and the virus infection time, create poisonous residues in the food chain. Understanding the growth stage critical for virus transmission can help greatly to undertake appropriate management measures to prevent virus transmission (Asare *et al.*, 2018).

### 2.6.2 Okra *leaf curl virus* (OkLCV)

*Okra leaf curl virus* is the name given to a complex of *Geminiviruses* of Family *geminiviridae,* genus *Begomovirus* affecting okra plants. The virus is characterised by twined isometric and circular single stranded DNA genomes. *Germinivirus* belonging to the genus *Begomovius* have monopartite or bipartite genomes, it is transmitted by white flies *Bemisia tabaci* Genn and infect dicotyledonous plants (Asare *et al*., 2014).

#### 2.6.2.1 Transmission

The virus is transmitted in a non-persistent manner by *Bemisia tabaci* Genn. and grafting, but cannot be transmitted by mechanical inoculation and also not by seed (Kumar *et al.,* 2014). Its host species include *Abelmoschus esculentus, Gossypium hirsutum,Solanum lycopersicum* L,*Nicotania tabacum* and *Urena lobata. Okra leaf curl virus* is one of the most common major diseases of okra and causes yield losses of up to 80% (Asare *et al.,* 2018).

#### 2.6.2.2 Symptoms and management

Symptoms of Okra leaf curl disease include leaf wrinkle, leaf curl, vein distortion, leaf yellowing; stunted growth and reduced yields (Askira, 2012). Symptoms of the virus include vein thickening, leaf crinkling and curling, vein enations, stunting and fewer or smaller pods and blisters may be seen in young and mature leaves, while the lamina may be torn and fewer and smaller fruits are produced (Alegbejo, 2015). It is quite important to effectively manage this virus disease in okra in order to improve yields and fruit quality. Information on the incidence and severity of these diseases is an important prerequisite for the development of appropriate and effective management strategies. Some management strategies are that okra should not be grown close to other *malvaceous* crops, the use of resistant cultivars, the use of

Cypermethrin to control whitefly vectors and integration of these control measures are effective in the management of the virus.

### 2.6.3 *Okra yellow mosaic virus* (OkYMV)

The major limiting factor for okra cultivation is the incidence of *Okra yellow mosaic virus* (OkYMV) which is transmitted by the whitefly *Bemisia tabaci* Genn (Fajinmi and Fajinmi, 2010). This disease is caused by a complex; consisting of monopartite *begomovirus*. *Okra yellow mosaic virus* belongs to the family *Germiniviridae* and is with small satellite DNA $\beta$component (Kumar *et al.,* 2014). This disease and its insect vector cause heavy losses to okra by affecting the quality and yield of the fruits (Fajinmi and Fajinmi, 2010). Infection of 100 % plants in a field is quite common and yield losses ranges from 50 to 94 % depending on the stage of the crop growth at which infection occurs (Kumar *et al.,* 2014).

#### 2.6.3.1 Symptoms

The initial symptoms on young leaves are irregular yellow intervienal areas. Clearing of the small veins starts near the margins, at various points about 15 to 20 days after infection. Thereafter, vein clearing develops into chlorosis. The newly developed leaves exhibit interwoven network of vein which encloses the green patches of the leaves (Kumar *et al.,* 2014). Fruits developing on infected plant have irregular yellow areas which follow a longitudinal alignment. As a result of the heavy infection, the fruits become malformed and sizes reduced (Fajinmi and Fajinmi, 2010). The fruits are mostly yellow, small, tough and fibrous (Kumar *et al.,* 2014). If the plants are infected within 20 days after germination, their growth is retarded; few leaves and fruits are formed. The extent of damage declines with

delay in infection of the pathogens, plant infected 50 to 65 days after germination suffer a loss 84 and 49 %, respectively.

#### 2.6.3.2 Management of *Okra yellow mosaic virus* (OkYMV)

The various control measures of *Okra yellow mosaic virus* have been widely studied which include treatments such as chemicals as well as plant extracts (Chaudhary *et al.,* 2016). Pathologists have tried to evaluate different plant extracts to manage this disease. Worldwide scientist preferred to adopt measures, which do not affect the human health. Different management practices are adopted to control plant virus diseases to overcome the okra production losses. The use of plant extracts to manage OkYMV disease and its vector is a cheap source as compared to all other expensive control measures. Effective and efficient pest control can be achieved by the use of chemicals but it is hazardous for the environment due to their toxicity levels. The spray of leaf extracts such as *Propos chilensis* and *Bougainviilea spectabilis* are very effective in managing *Yellow vein mosaic virus* of okra as these extracts increased the incubation period of the virus into the plants (Chaudhary *et al.,* 2016). However, it is also reported by Chaudhary *et al.,* (2016) that, the use of resistant and tolerant cultivars is very efficient and effective method to manage OYVMV disease and its vectors. Furthermore, plant extracts (Neem extracts at 5% concentration) have an efficient response for managing OYVMV disease incidence and to suppress whitefly population.

## 2.7 *Cucumber Mosaic Virus* (CMV)

*Cucumber mosaic virus* is the type species of the genus *Cucumovirus* in the family *Bromoviridae* (Mark *et al.,* 2014). *Cucumber mosaic virus* was first found in cucumber (*Cucumis sativus* L.) showing mosaic symptoms in 1934, hence the name *Cucumber mosaic*

(Zitter and Murphy, 2009). Since it was first recognised, it has been found to affect a great variety of other plants (Mark *et al.,* 2014). It consists of three spherical particles, each approximately 28 nm in diameter. The CMV genome consists of three single-stranded, messenger-senses RNA 1 with ~3,350 nucleotides, RNA 2 with ~3,050 nucleotides and RNA 3 with ~2,200 nucleotides (Alegbejo, 2015). Each RNA molecule is enclosed within a protective protein coat with each being distinct single spherical-shaped particle. Thus a mature CMV consists of three spherical particles, containing RNA 1, RNA 2 and RNA 3, but the particle may contain a fourth RNA strand, referred to as RNA 4 with~1,030 nucleotides, which encodes the coat protein gene and from which the CMV coat protein is produced (Mark *et al.,* 2014). This type of translational strategy, referred to as sub-genomic RNA, consists of a separate strand of RNA produced during replication. While CMV RNA 3 contains the coat protein gene, the gene is only translated to produce the coat protein from its sub-genomic strand (Zitter and Murphy, 2009). *Cucumber mosaic virus* exists in numerous strains that differ somewhat in their hosts, in the symptoms they produce, in the ways they are transmitted, and in other properties and characteristics (Mark *et al.,* 2014).

The virus is systemic in its host. Older tissues and organs that develop prior to infection usually are not affected by the virus, but newer cells and tissues that develop after infection may be affected with varying severity (Zitter and Murphy, 2009). The concentration of the virus increases as the plants get older for several days following inoculation, then decreases until it levels off or the plant dies (Mark *et al.,* 2014). Infectivity is lost within a few days, and in some instances, hours. *Cucumber mosaic virus* is relatively unstable when it is in plant extracts or sap and also unable to withstand temperatures in excess of 70° C for 10 minutes (Mark *et al.,* 2014).

### 2.7.1 Symptoms

Symptoms of *Cucumber mosaic virus* infection in okra consist of leaf curl, blistering, chlorotic mosaic patterns on leaves, stunted growth, leaf distortion, mottling and colour breaking of fruits (Zitter and Murphy 2009). Symptoms are most obvious on the leaves and pod infection and subsequent loss is greatest when plants are infected before bloom. Early infection on plants may result in few pods, because CMV causes flower abortion and abnormal development such that the pods are mostly curved, mottled and reduced in size. Plants may recover and resume normal growth with limited yield loss if infected after bloom. The virus causes a wide range of symptoms depending on host, age of the plant, virus strain and environmental conditions. The disease is sometimes referred to as "shoestringing" because of the effect on young leaves which develop a narrow, elongated, tendril-like appearance (Mark *et al.,* 2014). Other common symptoms in vegetable plants include deformity, wrinkling, twisting, curling, yellowing or chlorosis and mosaic or mottling (Zitter and Murphy, 2009).

### 2.7.2 Transmission

*Cucumber mosaic virus* is transmitted primarily by aphids, and also by seed, cucumber beetles, parasitic plants, humans and mechanically. It is transmitted by more than 60 species of aphid, notably *Aphis gossypii* Glover and *Myzus persicae* Sulzer in a non-persistent manner, and is readily transmissible through plant sap (Mark *et al.,* 2014). *Cucumber mosaic virus* can be acquired by aphids in 5-10 seconds and transmitted in less than 1 minute. The ability of CMV to be transmitted declines after about 2 minutes and is usually lost within 2 hours (Mark *et al.,* 2014). In addition, some isolates can lose their transmissibility by one aphid species but retain their transmissibility by another. Transmission through seed occurs

to varying degrees in 19 host species, including some important weeds. Dissemination and persistence in weed seeds may be important in the epiphytology of CMV (Mark *et al.,* 2014). The virus can be transmitted between plants by parasitic plants that are able to host and transmit CMV. At least 10 species of *Cuscuta* or dodder are able to multiply and transmit CMV.

*Cucumber mosaic virus* overwinters in many perennial weeds, flower and crop plants. Perennial weeds such as white cockle, wild ground cherry, horse nettle, milkweed, ragweed, pokeweed, nightshade, and various mints harbour the virus in their roots during the dry season and carry it to their top growth in the growing season from which aphids transmit it to susceptible crop plants (Mark *et al.,* 2014). Once few plants have become infected with CMV, insect vectors and humans especially during cultivating and handling the plants readily spread the virus to healthy plants.

### 2.7.3 Host range

*Cucumber mosaic virus* has a wide range of hosts and attacks a great variety of vegetables, ornamentals, and as many as 1,200 species belonging to hundreds of plant families (Zitter *et al.,* 2009). This wide variety of hosts means the disease can be a persistent problem in both agriculture and non-agricultural settings. Among the most important vegetables affected by virus are peppers (*Capsicum annuum* L.) cucurbits, tomatoes (*Solanum lycopersicum* L), and bananas (*Musa* spp. L.). Other vegetable hosts include: cucumber (*Cucumis sativus* L.), muskmelon (*Cucumis melo* L.), squash, (*Cucurbita maxima* Duc.) spinach (*Spinach oleracea* L.), celery (*Apium graveolens* Mill.), and watercress (*Nastrutium nasturtium-aquaticum* L H. Karst) (Mark *et al*., 2014). Alegbejo, 2015 stated that beet (*Beta vulgaris* L.), sweet potato (*Ipomoea batatas* (L.) Lam.), turnip (*Brassica rapa* L.), chayote (*Sechium edulis* Jacq.Sw.),

watermelon (*Cirtrullus lanatus* L.), pumpkin (*Cucurbit apepo* Duc) and parsley (*Petroesslinum crispum* Mill. Fuss) loofah (*Luffa cylinderica* (L.) Roem) rtichoke (*Cynara cardunculus* L.) are also important host of the CMV.

Ornamental hosts include China aster (*Callistephus chine* L. Nees), Delphinium (*Delphinium nuttallianum* L.), salvia (*Saliva officinalis* L.), geranium (*Geranium renardii* L.), gilia (*Gilia chilleifoli* Benth.), gladiolus *(Gladiolus angustus* L.), heliotrope (*Heliotropium europaeum* L.), hyacinth (*Hyacinth orrientalis* Tourn.), larkspur (*Consolida armeniaca* DC), lily (*Lilium martago* L.), Marigold (*Calendula officinalis* L.), Morning glory (*Ipomoea purpurea* {L.}Roth), masturtium (*Nasturtium microphyl* (Boenn.), periwinkle (*catharathus roseus* L.) phlox (*Phlox diffusa*Benth.), snapdragon (*Antirrhium majus* L.), tulip (*Tulipal inifolia* Regel), and (*Zinnia peruviana* L.) (Alegbejo, 2015).

### 2.7.4 Detection and identification of *Cucumber mosaic virus*

Identification of *Cucumber mosaic virus* is on the basis of biological properties or external symptoms, host range, mode of transmission, virus stability, inclusion bodies and virus morphology, serological reaction and molecular characters (Mark *et al.,* 2014). Detection of *Cucumber mosaic virus* is based on Bioassay, Double Antibody Sandwich Enzyme-Linked Immunosorbent Assay (DAS-ELISA), Electron Microscopy, Immunosorbent Electron Microscopy, Cytopathology, Reverse Transcription Polymerase Chain Reaction (RT-PCR) and Sequencing (Mark *et al*., 2014). The simplest method used in detecting CMV is the lateral flow assay which is more practical and least expensive for small-scale use. Other serological methods may be more efficient on larger scale, such as ELISA and isothermal

amplification method such as Loop-Mediated Isothermal Amplification (LAMP) (Mark *et al.,* 2014).

### 2.7.5 Management of *Cucumber mosaic virus*

The first steps to manage *Cucumber mosaic virus* are detection, identification and determining the pathogen characteristics. Control of CMV weed hosts near cultivated fields is often successful in reducing the incidences of CMV in cucumber and celery, and is likely beneficial in other crop fields as well (Mark *et al.,* 2014). Perennial weeds should be eradicated from around greenhouses, garden and fields to eliminate possible sources of CMV. Apart from the aphid vectors; CMV is easily transmitted on garden tools and gardeners' fingers. Other measures are by avoiding avoid handling healthy plants after working with suspected infected ones until tools or hands have been washed with soapy water and destroying suspected plants promptly to reduce the risk of transmission.

# CHAPTER THREE

# 3.0 MATERIALS AND METHODS

## 3.1 Description of the Study Location

The study was conducted in Niger State, Nigeria. Niger State is located in the Southern Guinea Savanna Agro-ecological zones of Nigeria. It lies between Latitude 3.20°North and Longitude 8 and 11.3° East. It has a total land area of 83,266,779 square kilometers or about 8.3 million hectares which represent 8 % of the total land area of Nigeria with about 85 % of the land is arable. The State has an average rainfall of between 1,100 mm to 1,600 mm which is distributed between April and early October reaching its peak at September of each year. Temperature ranges between 35°C and 37° C and the relative humidity of between 40 % and 80 % (Adeboye *et al.,* 2011). The soils, like most soils are characterized by flooded plains with considerable variation which has little hazards. The soils have a good water holding capacity which originated from basement complex rocks and are generally classified as Alfisols (Adeboye *et al.,* 2011).

## 3.2 Determination of Okra Viruses

Six Local Government Areas (LGAs) in Niger State namely Borgu,Bosso, Gbako, Mashegu, Mokwa and Paikoro were surveyed for incidence and severity of okra viruses (*Okra mosaic virus* and *Cucumber mosaic virus).* These LGAs were selected because they are known for high level of okra cultivation.

## 3.3 Survey and Sample Collection

A total of 24 okra fields were surveyed in six LGAs in the State for okra viruses in the months of September to October, 2019 cropping season. The exact coordinates of each farm

were captured using the Global Positioning System (GPS) equipment (GPS- 4300; Ethrex Garmin GPS, Taiwan). Photographs of all samples were also taken. Detailed information including date and time of visit, farm size, cropping system, crops in neighbouring fields, insect pests and disease management strategies by the farmers were recorded using a well-structured questionnaire. Depending on the symptom types, between 1 and 5 leaves were collected from each field, from okra plants exhibiting virus and virus-like symptoms giving a total of120 leaf samples. The leaves were preserved in vial bottles containing silica gel to maintain viability and inherent drying before they were transferred to the laboratory for indexing.

## 3.4 Virus Identification

### 3.4.1 Source of polyclonal antibodies (PAbs)

*Okra mosaic virus* and *Cucumber mosaic virus* polyclonal antisera were obtained from Leibniz-Institute, DSMZ- Deutsche Sammlung von, Mikroorganismen und Zeillkulturen GmbH, Germany.

### 3.4.2 Double antibody sandwich enzyme link immunosobent assay (DAS-ELISA)

The samples collected from field survey were subjected to Double Antibody Sandwich Enzyme-Linked Immunosorbent Assay (DAS-ELISA) according to manufacturer's instructions. The PAbs were diluted in coating buffer ($Na_2CO_2$ 1.59 g, NaHCO 2.93 g and sodium diethyl dithiocarbamate 10 g in 1 litre of distilled water with pH adjusted to 9.6) at a rate of 23.2 micro litre (µL) and 200 µL was added to each well of the microtitre plate. The plates were incubated at 37 $^{\circ}$C for 2 hours, and thereafter washed with phosphate buffered saline- Tween (PBS- T) (PBS-T: $Na_2HPO$ 22 g, $KH_2$, $PO_4$ g, KCl 4 g, NaCl 160 g and 10 ml

of Tween- 20, made up to 2 litres with distilled water, at pH 7.4). After blotting on tissue paper, the plates, 200 µL of conjugate enzyme was added. The plates were incubated again at 37 °C for 2 hours and then washed thrice with PBS- Tween. Two hundred microlitres aliquots of freshly prepared substrate made of 1 mg/ ml para – nitrophenyl –phosphate in substrate buffer (10 % diethanolamine in distilled water, at pH 9.8) was added to each well, covered and incubated at 37 °C for 60 minutes in the dark to obtain clear reaction. Finally, the results were assessed through spectrophotometric measurement of absorbance at 405 nm, using a microplate reader (MRX, Dynex Technologies, Inc., USA). Samples were considered positive when the optical density reading was at least twice that of the mean for the negative controls.

## 3.5 Evaluation of Okra Cultivars against *Cucumber Mosaic Virus*

### 3.5.1 Experimental site

The experiment was conducted in the screenhouse at the Teaching and Research Farm of the Department of Crop Production, Federal University of Technology Minna, Niger State Nigeria.

### 3.5.2 Multiplication of virus inoculum

The CMV-positive isolates from field survey were multiplied in a local okra variety (Clemson spineless) by mechanical inoculation as follows. Infected leaf samples were ground in inoculation buffer pH 7.2 (0.1M $NaHPO_4$, 0.1M $K_2PO_4$, 0.01M Ethylene diame tetra acetic acid, and 0.001M L-cysteine). One microlitre of β-mecaptor ethanol was added to the extract and carborundum powder (600-mesh) was rubbed on the upper leaf surface before sap

application. Symptomatic leaves resulting from the plants eliciting symptoms CMV disease were used for inoculation of the okra genotypes.

### 3.5.3 Treatments and experimental design

Four genotypes of okra were arranged on iron benches in a Completely Randomized Design (CRD) with three replications at the screenhouse. Each cultivar was evaluated in polypots of 23 cm in diameter, 30 cm deep (Plate 3.1).

### 3.5.4 Source of seeds

Okra genotypes designated as: OKR_19_01, OKR_19_02, OKR_19_03 and OKR_19_04 were sourced from the Genetic Resource Unit of the National Horticultural Research Institute (NIHORT) Ibadan, Oyo State, Nigeria.

### 3.5.5 Soil sterilization

The top soil used was collected from the Teaching and Research Farm of the Department of Crop Production, Federal University of Technology Minna, Niger State, Nigeria. It was sterilised using trough method as described by Handiseni *et al*, (2010). The trough method consisted of upper and lower pieces. The upper piece with perforation at the bottom served as the soil container, while the lower piece contained the water. The lower piece was positioned on a metal stand (tripod stand) and filled with water, the upper piece was placed on top of the lower part covered with thick jute sack followed by a moderately tight-fitting lid. Woods were then set in between the metal stand and fire set on the woods. The steam generated passed through the perforated bottom of the top piece of the mechanism to sterilise the soil to the temperature of 100˚C.

Plate 3.1: Experimental layout of the screenhouse trial

### 3.5.6 Crop establishment and mechanical inoculation

Screening of okra genotypes for CMV disease resistance was conducted in a screenhouse. Seeds were sown in poly pots (23 cm in diameter, 30 cm deep) containing sterilized soil of 6 kg each. The seedlings were thinned to two stands per pot at eight days after sowing. Inoculations were carried out at 10 DAS. Extract preparation and inoculation procedure were carried out as described in 3.5.2 above. Uninoculated plants of each genotype served as control.

## 3.6 Data Collection

### 3.6.1 Disease incidence

Disease incidence was assessed as percentage of the plants showing typical virus symptoms after inoculation. It was observed at 1, 2 and 3 weeks after inoculation (WAI) and expressed in the following relation:

$$\text{Disease incidence} = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$$

### 3.6.2 Disease severity

Disease severity was assessed at 3, 4 and 5 WAI, as percentage of leaf surface exhibiting virus symptoms following the modified rating scale of Asare *et al.* (2018), where:

1 = No symptoms on leaf surface,

2 = Mosaic symptom on 25 % of top leaf surface,

3 = Mosaic symptom on 50 % of top leaf surface,

4 = Mosaic symptom on 75 % of top leaf surface with curling and slight stunting of plant and

5 = Mosaic symptom on >75 % of top leaf surface, severe curling and stunting.

### 3.6.3 Plant height

Plant height was obtained by measuring the plant from the soil surface to the tip of the topmost leaf of the plant (cm) using a metre rule. The measurement was recorded at 2, 4 and 6 weeks after inoculation (WAI). The average plant height was calculated for each treatment and expressed in centimetres (cm).

### 3.6.4 Number of leaves per plant

This was done by counting the number of leaves on each plant in the pot. This was evaluated at 2, 4 and 6 WAI

### 3.6.5 Number of days to flowering

Days to flowering was determined by counting days from date of sowing to the opening of the first flower.

### 3.6.6 Number of fruits per plant

This was determined at maturity by counting number of fruits on the plant in the pot.

### 3.6.7 Fruit length

The fruit length was measured after harvest for each plant using string and metre rule. The mean was calculated and expressed in centimetres (cm)

### 3.6.8 Fruit weight per plant

The fruit weight per plant was determined after harvesting by weighing all the fruits harvested from each pot with an electronic top loading Mettler weighing balance (METTLER PM2000, Greifensee, Switzerland) and expressed in gram (g).

### 3.6.9 Fruit diameter

Fruit diameter was assessed after harvesting of the fruits by measuring the circumference of the fruits using a measuring tape. The mean was calculated and expressed in centimetres (cm)

## 3.7 Statistical Analysis

The data collected from field survey were subjected to descriptive statistics, while data on plants responses to CMV disease were subjected to analysis of variance (ANOVA) at $p$=0.05. Genotypes' means were separated using the Least Significant Difference (LSD). Statistical analysis was performed using Statistical Analysis System (SAS, 2008).

# CHAPTER FOUR

## 4.0 RESULTS AND DISCUSSION

### 4.1 Cropping History of the Farms Surveyed for Okra Viruses

Results obtained from the survey on the frequency distribution of okra sources of seeds cultivated in the selected LGAs of Niger State in the 2019 cropping season (Fig 4.1) showed that farmer obtained their seeds from different sources. Out of the 24 responses, 20 farmers obtained their cultivated seeds from their previous harvests, followed by 3 farmers that obtained their seeds from friends and the least response of 1 which was obtained from Agro-stores. However, there were no responses for seeds obtained from Agricultural Development Projects (ADPs) and Research Institutes (Fig 4.1).

Frequency distribution of okra cultivar interests cultivated in selected LGAs of Niger state in the 2019 cropping season (Fig 4.2) showed that farmers cultivate okra crop with the interest of genetic characteristics and market values attributed to a particular cultivar. Early maturing and high yielding cultivars had the highest responses of 8 each. However, cultivars selected for marketability purposes had 6 responses, late maturing cultivars resulted in the lowest responses of 2 (Fig 4.2).

Intercropping preferences among okra cultivated farmers in selected LGAs of Niger State in 2019 cropping season (Fig 4.3) showed that the indigenous farmers in Niger State however prefers intercropping okra with other crops. Intercropping preferences taken showed that 6 farmers intercropped okra with pepper and soyabean. There were 5 responses for okra intercropped with garden egg, 3 responses for okra intercropped with maize and okra

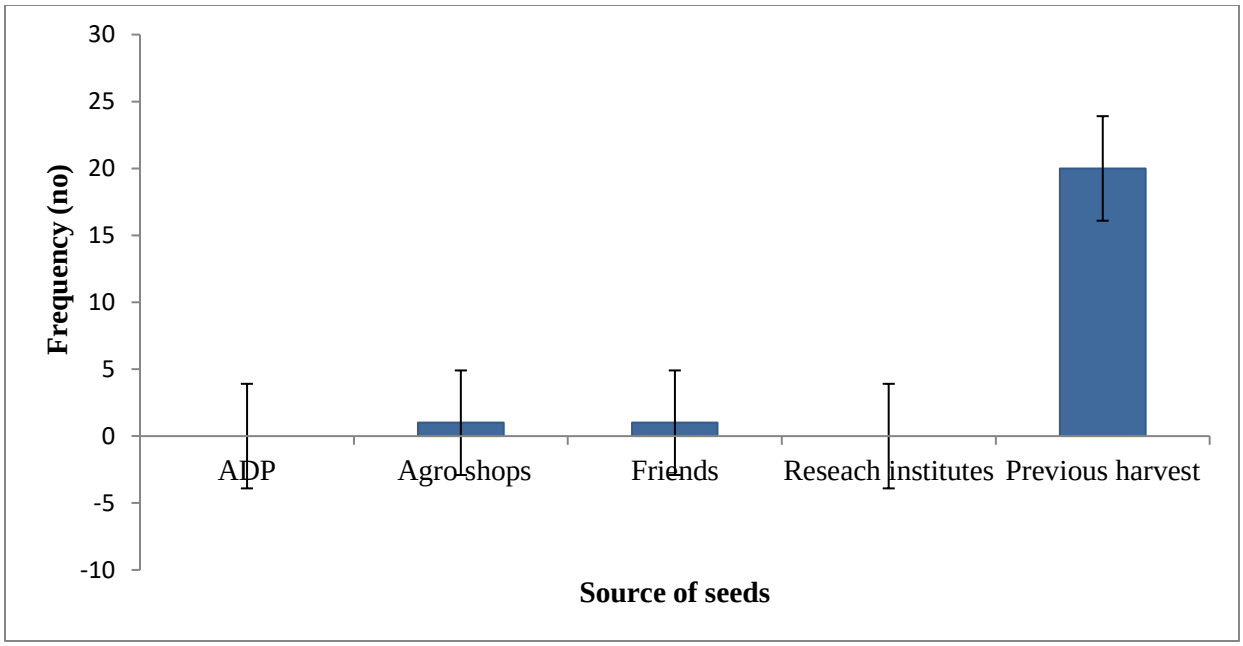


Figure 4.1.Frequency distribution of sources of okra seeds cultivated in selected Local Government Areas of Niger State in the 2019 cropping seasons

ADP - Agricultural Development Project

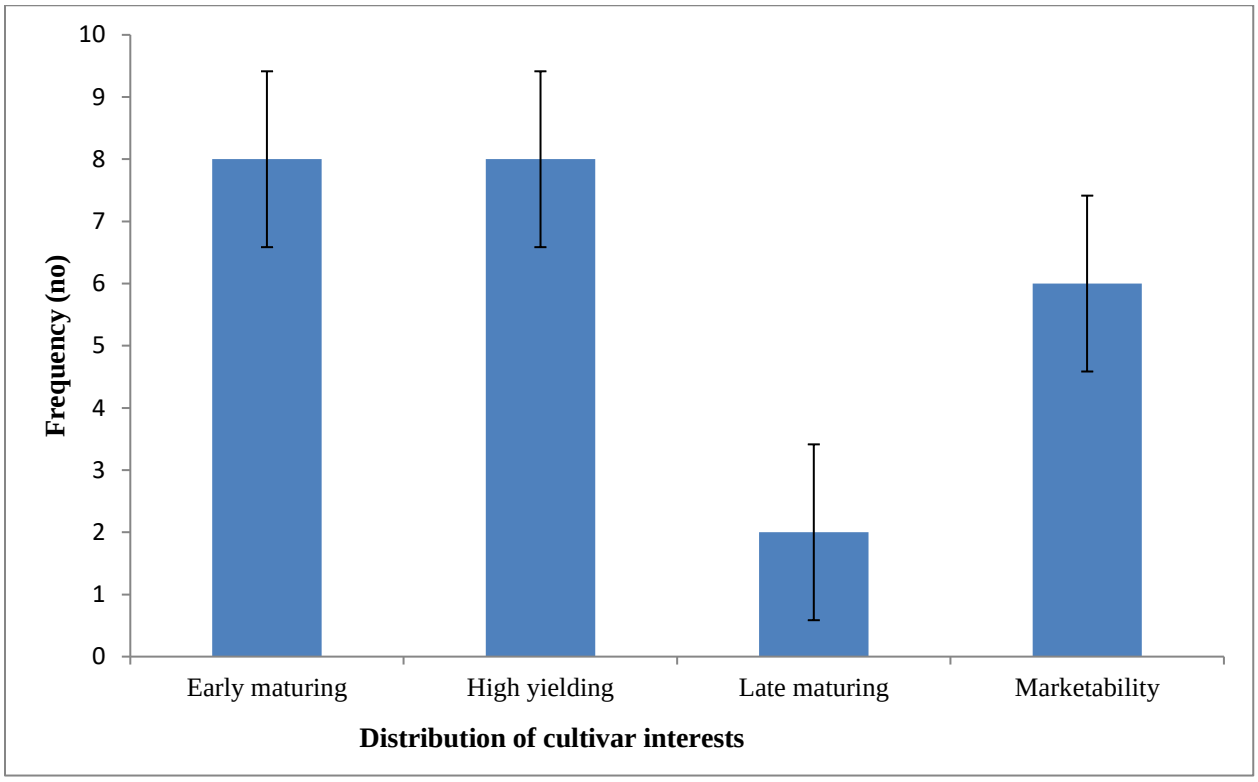


Figure 4.2. Frequency distribution of okra cultivar interest cultivated in selected Local Government Areas of Niger State in the 2019 cropping season

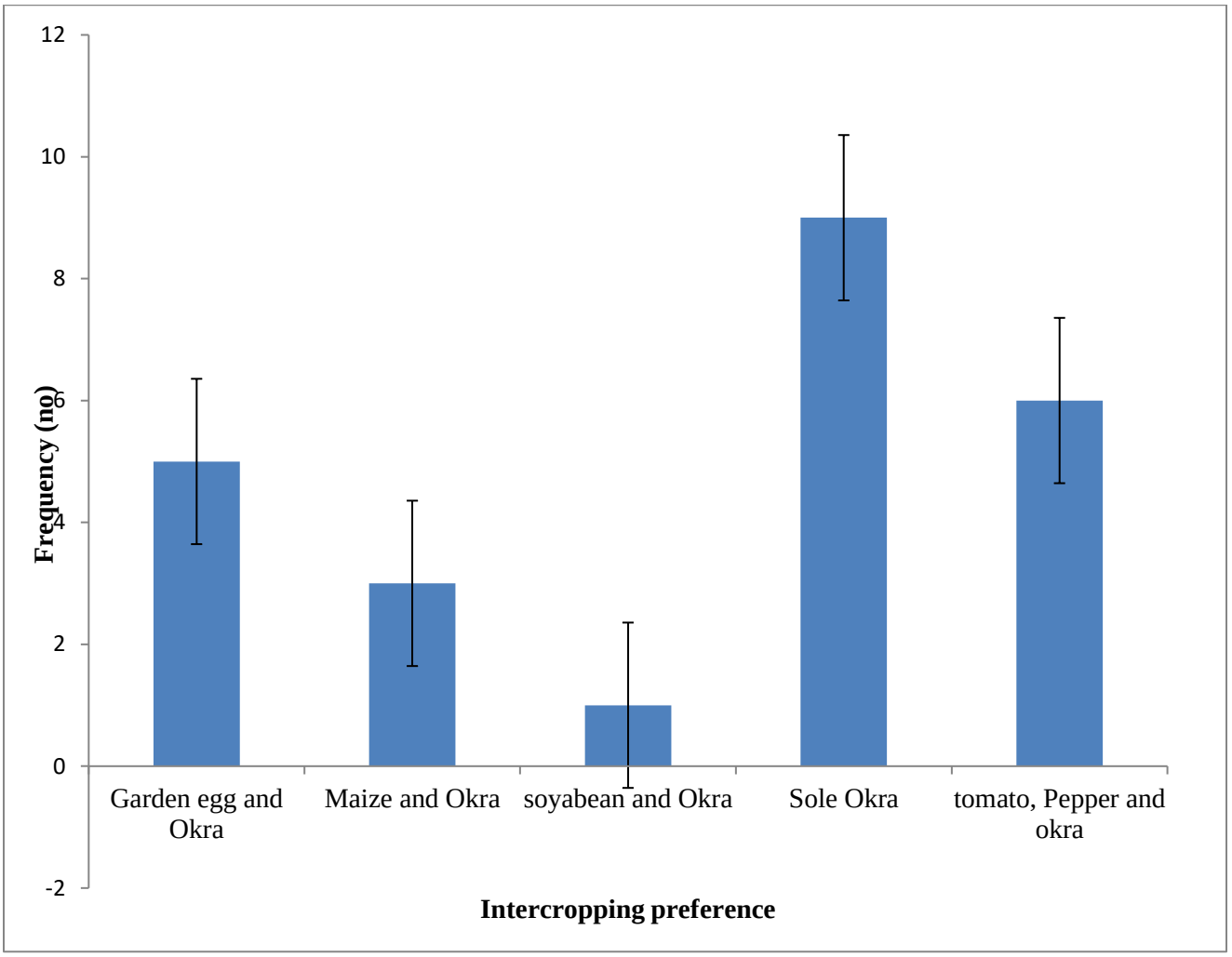


Figure 4.3.Intercropping preferences by okra farmers in selected local Government Areas of Niger State in 2019 cropping season

whiles oyabean and okra recorded the least response of 1. However, Sole okra had the highest response of 9 (Figure 4.3).

Frequency distribution of cultivated of okra cultivars in selected LGAs of Niger State in the 2019 cropping season shown in Figure 4.4. Farmers in the studied areas mostly cultivated locally adopted cultivars. Results from this study showed that cultivars “Kpammi Bokungi” and “Saga” gave the highest responses of 5 each, followed by “Foshiba” which had 3 responses. Cultivar “Cocurafegi”, “Danrani” and “Dowe” had 2 responses each, while the least responses of 1 was observed in cultivar “Damina”, “Kpammi Bie” and “Pkeeten (Figure 4.4).

Frequency distribution on purpose of cultivated okra cultivars in selected LGAs of Niger State in the 2019 cropping season (Figure 4.5) showed that farmers in the surveyed areas of Niger state cultivate okra mostly for consumptions and sales. The result revealed that for the purpose of cultivation, the highest response was observed for consumption and sales with 18 responses followed by purpose of sales alone (4 responses). The lowest response observed in this study was however in consumption purpose only (2 responses) (Figure 4.5). For the purpose of cultivated hectares, the highest frequency recorded was with hectare of between 0.1 and 0.5 with 23 responses and the least was between 0.6 and 1.0 (1 response) (Figure 4.6). However, no response was recorded for cultivated hectares above 1. Response to fertilizer preferences among okra farmers is shown in Figure 4.7, 12 farmers preferred okra production with zero fertilizer application. This was followed by the use of organic fertilizer with 8 responses and least response was recorded in NPK fertilization preference with 4 responses (Figure 4.7).

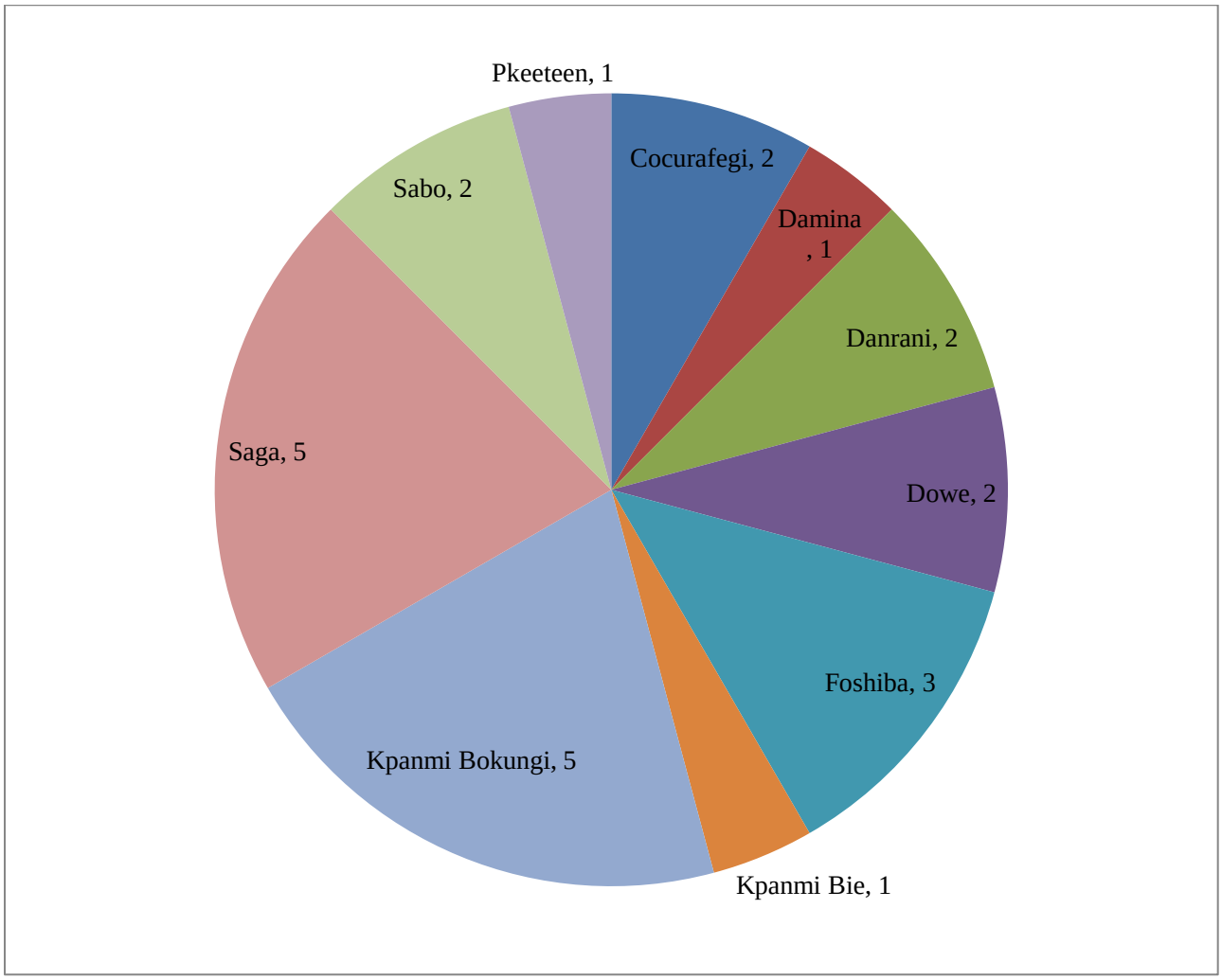


Figure 4.4. Frequency distribution of cultivated okra cultivars in selected Local Government Areas of Niger State in the 2019 cropping season

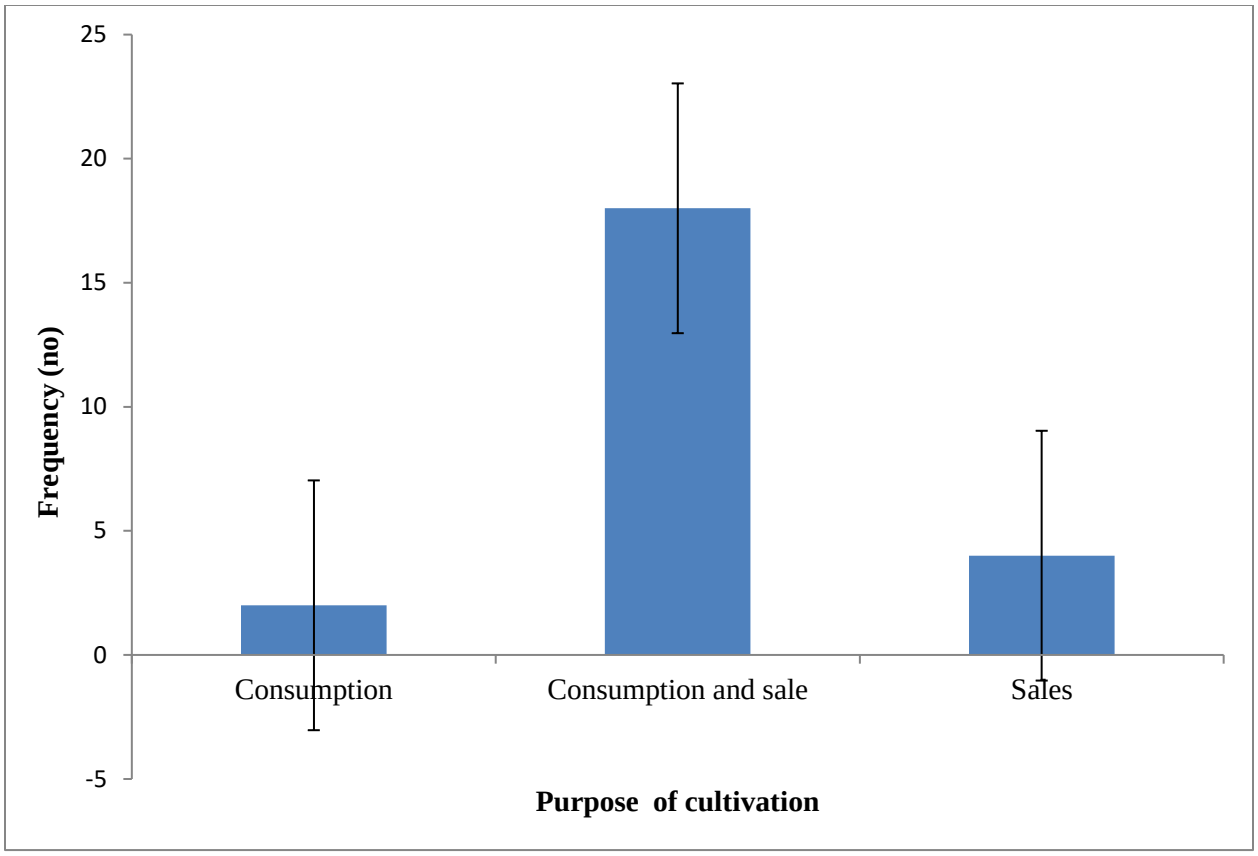


Figure 4.5. Frequency distribution on purpose of okra cultivation in selected Local Government Areas of Niger State in the 2019 cropping season

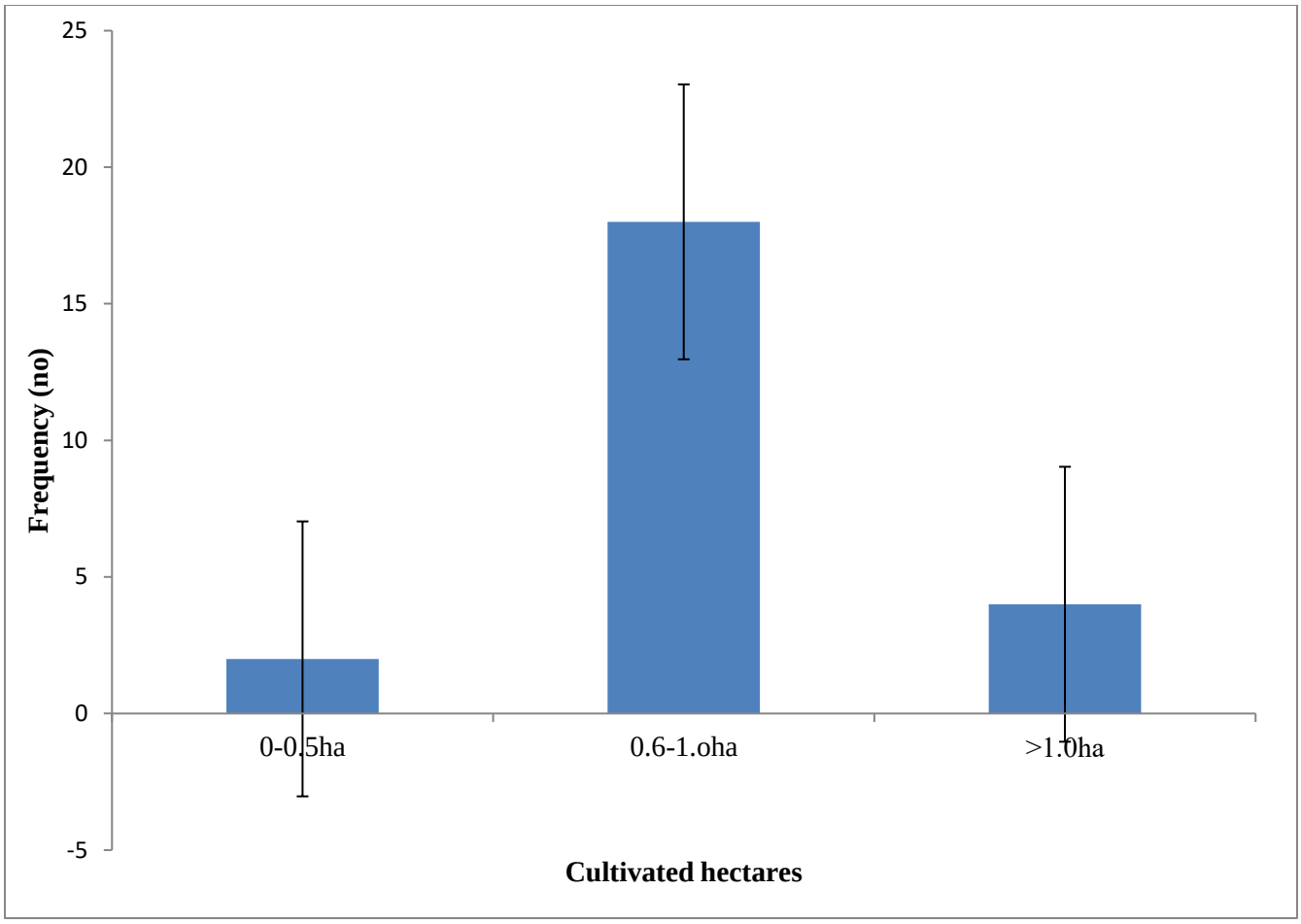


Fig 4.6. Frequency distribution of cultivated okra farmlands in selected Local Government Areas of Niger State in the 2019 cropping season

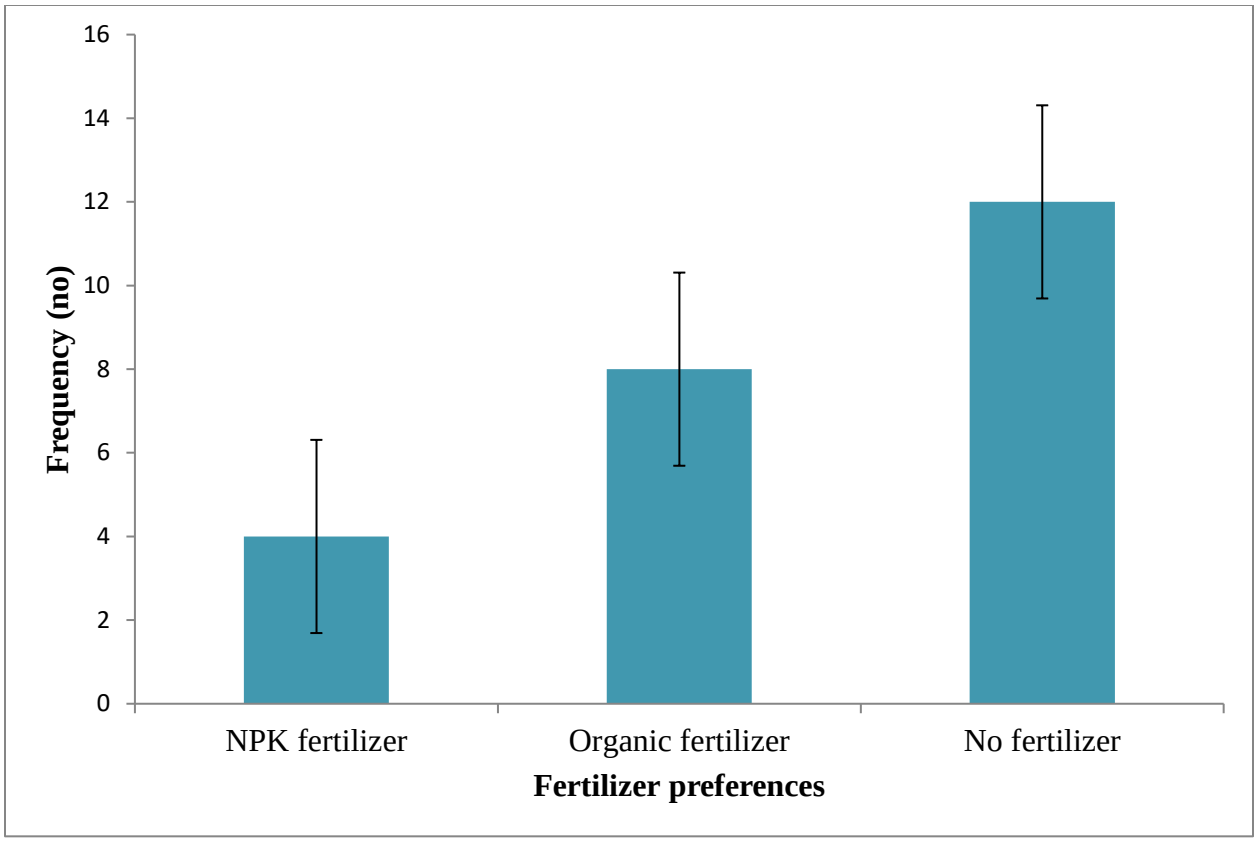


Figure 4.7. Fertilizer use preferences among okra farmers in selected Local Government Areas of Niger State in the 2019 cropping season

### 4.1.2 Distribution of *Cucumber mosaic virus* and *Okra mosaic virus* in the Study Area

*Cucumber mosaic virus* and OkMV were detected during the survey, with the former being more prevalent. A total of 11 samples (9.2 %) reacted positively with CMV PAb (Table 4.1). Incidence of CMV disease was highest in Borgu (3.3 %) and Mokwa LGAs (3.3 %), followed by Paikoro LGA (1.7 %) while an incidence of 0.8 % was found in Gbako LGA. On the other hand, none of the samples from Bosso and Mashegu reacted positively with CMV PAb. In Borgu LGA, CMV disease was found at Dogongari, Gadaoli, Fakun and Malele. In Gbako LGA, the virus was detected only at Emisomma community. In Mokwa LGA, the disease occurred at Government Teachers College, Waabi, Wuya-Kede and Rail Station. The two CMV-positive samples in Paikoro LGA came from Jankpan and Jyipe communities. On the other hand, only 2 samples (1.7 %) tested positive for OkMV disease. These were found at Gidan-Mugoro and Edokota, in Bosso and Gbako LGA, respectively (Table 4.2).

### 4.1.3. Disease Incidence and Severity in Okra Genotypes Infected with *Cucumber mosaic virus*

All the plants inoculated with CMV elicited disease symptoms. Symptom manifested at ten days after inoculation (DAI). The symptoms started as mild mottling of the topmost leaves, blistering, curling and some leaves exhibited yellow colouration while the uninoculated plants were apparently healthy (Plate II). Disease incidence varied significantly ($p<0.05$) among the inoculated plants (Table 4.3). At 1 WAI disease incidence ranged from 33.1 to 100 %, with the highest incidence (100 %) observed on “OKR_19_01” and “OKR_19_02”. This was followed by “OKR_19_03” and “OKR_19_04” with 33.3 and 33.1 % level of infection, respectively. The difference in disease incidence between “OKR_19_03” and “OKR_19_04” genotypes was not significant ($p>0.05$). At 2 and 3 WAI, 100 % disease

incidence was observed on “OKR_19_01” and “OKR_19_02”100 %, whereas “OKR_19_03” and “OKR_19_04” exhibited an incidence of 88.9 and 66.6%, respectively. There were no significant differences in disease severity among the genotypes across the study period. At 3, 4 and 5 WAI, disease severity was consistently lowest on “OKR_19_03” with a mean score of 2 (Table 4.3). The genotype “OKR_19_04” maintained a severity score of 2.3 throughout the period of evaluation. Conversely, a mean severity of 2.3 was observed on “OKR_19_01” and “OKR_19_02” at 3 and 4 WAI and the value decreased to a score of 2 at 5 WAI.

### 4.1.4. Effects of *Cucumber mosaic virus* infection on agronomic performance of okra

#### 4.1.4.1. Effect of *Cucumber mosaic virus* infection on plant height

*Cucumber mosaic virus* disease impaired plant height regardless of the genotypes while uninoculated plants exhibited normal growth and vigour. At 2 WAI, the heights of virus-infected plants varied between 2.9 cm in “OKR_19_04” to 4.0 cm in “OKR_19_01” but the height differences were not significant (Table 4.4). Uninoculated plants’ heights ranged significantly from 4.9 cm (OKR_19_02 and OKR_19_04) and 5.7 cm (OKR_19_01). However, at 4 WAI, the trend of plant height took another dimension. The CMV-infected plants of OKR_19_01 were the shortest (5.3 cm) but the value obtained was not significantly different from the mean height of 5.8 cm in “OKR_19_02” and 5.7 cm from “OKR_19_04”; the CVM-infected plants of “OKR_19_03” which exhibited a mean height of 7.9 cm were the tallest. Although the heights of uninoculated plants ranged from 7.9 cm (OKR_19_04) to 12.1 cm (OKR_19_03), the differences observed were not significant. At 6 WAI, the virus-infected plants of “OKR_19_03” with a mean height of 17.3 cm were the tallest. Infection of “OKR_19_04” plants with CMV resulted in a mean height of 9.6 cm which was not

Table 4.1: Geographical details of okra farms and the distribution of *Cucumber mosaic* and *Okra mosaic viruses* from selected Local Government Areas of Niger State in 2019

| LGA | Location | Latitude ($^{O}$N) | Longitude ($^{O}$E) | Elevation (m) | No. of Sample | CMV Status | OkMV Status |
|---|---|---|---|---|---|---|---|
| Borgu | Dogongari | 10.088 | 5.588 | 162 | 5 | + | - |
| | Gadaoli | 10.011 | 4.591 | 254 | 5 | + | - |
| | Fakun | 9.692 | 5.522 | 204 | 5 | + | - |
| | Malele | 10.184 | 5.459 | 288 | 5 | + | - |
| Bosso | Beji | 9.636 | 6.330 | 273 | 5 | - | - |
| | Dama | 9.554 | 6.479 | 214 | 5 | - | - |
| | Gidan-Mungoro | 9.570 | 6.486 | 238 | 5 | - | + |
| | Garatu | 9.484 | 6.437 | 209 | 5 | - | - |
| Gbako | Edokota | 9.099 | 6.000 | 155 | 5 | - | + |
| | Emisomma | 9.148 | 6.013 | 149 | 5 | + | - |
| | Gbarifu | 9.126 | 5.923 | 146 | 5 | - | - |
| | Mungorota | 9.144 | 6.020 | 139 | 5 | - | - |
| Mashegu | Baban Rami | 10.525 | 5.738 | 346 | 5 | - | - |
| | Gidan-Kanbari | 9.719 | 5.037 | 145 | 5 | - | - |
| | Ibbi | 9.850 | 6.368 | 121 | 5 | - | - |
| | Kawo | 9.787 | 5.716 | 215 | 5 | - | - |
| Mokwa | G.T.C | 9.545 | 5.150 | 206 | 5 | + | - |
| | Rail Station | 9.468 | 5.062 | 168 | 5 | + | - |
| | Waabi | 9.699 | 5.186 | 163 | 5 | + | - |
| | Wuya-Kede | 9.135 | 5.921 | 168 | 5 | + | - |
| Paikoro | Gbadna | 9.431 | 6.638 | 321 | 5 | - | - |
| | Gbaita | 9.424 | 6.619 | 301 | 5 | - | - |
| | Jankpan | 9.411 | 6.633 | 285 | 5 | + | - |
| | Jyipe | 9.415 | 6.631 | 285 | 5 | + | - |

LGA = Local Government Area; CMV = *Cucumber mosaic virus*; OkMV = *Okra mosaic virus*; G.T.C= Government Teachers College

Table 4.2: Serological detection of okra farms and distribution of *Cucumber mosaic* and O*kra mosaic* viruses concentration from some Local Government Areas of Niger State in 2019

| LGA | Location | ODV | CMV | ODV | OkMV | LGA | Location | ODV | CMV | ODV | OkMV |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Borgu | Dogongari1 | 0.156 | - | 0.142 | - | Bosso | Beji1 | 0.114 | - | 0.00 | - |
| | Dogongari2 | 0.471 | + | 0.132 | - | | Beji2 | 0.109 | - | 0.13 | - |
| | Dogongari3 | 0.224 | - | 0.124 | - | | Beji3 | 0.11 | - | 0.133 | - |
| | Dogongari4 | 0.142 | - | 0.128 | - | | Beji4 | 0.107 | - | 0.159 | - |
| | Dogongari5 | 0.273 | - | 0.124 | - | | Beji5 | 0.135 | - | 0.143 | - |
| | Gadaoli1 | 0.362 | - | 0.138 | - | | Dama1 | 0.149 | - | 0.156 | - |
| | Gadaoli2 | 0.211 | - | 0.127 | - | | Dama2 | 0.14 | - | 0.168 | - |
| | Gadaoli3 | 0.284 | - | 0.144 | - | | Dama3 | 0.127 | - | 0.156 | - |
| | Gadaoli4 | 0.25 | - | 0.129 | - | | Dama4 | 0.135 | - | 0.164 | - |
| | Gadaoli5 | 0.458 | + | 0.128 | - | | Dama5 | 0.145 | - | 0.136 | - |
| | Fakun1 | 0.214 | - | 0.133 | - | | Garatu1 | 0.13 | - | 0.14 | - |
| | Fakun2 | 0.18 | - | 0.00 | - | | Garatu2 | 0.114 | - | 0.157 | - |
| | Fakun3 | 0.251 | - | 0.133 | - | | Garatu3 | 0.133 | - | 0.148 | - |
| | Fakun4 | 0.238 | - | 0.134 | - | | Garatu4 | 0.115 | - | 0.136 | - |
| | Fakun5 | 0.163 | - | 0.136 | - | | Garatu5 | 0.117 | - | 0.00 | - |
| | Malale1 | 0.347 | - | 0.139 | - | | Gidan-Mungoro1 | 0.135 | - | 0.263 | + |
| | Malale2 | 0.482 | + | 0.146 | - | | Gidan-Mungoro2 | 0.134 | - | 0.169 | - |
| | Malale3 | 0.335 | - | 0.125 | - | | Gidan-Mungoro3 | 0.162 | - | 0.133 | - |
| | Malale4 | 0.267 | - | 0.115 | - | | Gidan-Mungoro4 | 0.121 | - | 0.144 | - |
| | Malale5 | 0.471 | + | 0.127 | - | | Gidan-Mungoro5 | 0.127 | - | 0.157 | - |
| Gbako | Edokota1 | 0.164 | - | 0.203 | - | Mashegu | Babanrami1 | 0.109 | - | 0.156 | - |
| | Edokota2 | 0.203 | - | 0.138 | - | | Babanrami2 | 0.106 | - | 0.149 | - |
| | Edokota3 | 0.174 | - | 0.247 | + | | Babanrami3 | 0.11 | - | 0.14 | - |
| | Edokota4 | 0.191 | - | 0.148 | - | | Babanrami4 | 0.099 | - | 0.00 | - |
| | Edokota5 | 0.135 | - | 0.145 | - | | Babanrami5 | 0.112 | - | 0.138 | - |
| | Emisomma1 | 0.147 | - | 0.135 | - | | Gidankambari1 | 0.128 | - | 0.149 | - |
| | Emisomma2 | 0.161 | - | 0.00 | - | | Gidankambari2 | 0.118 | - | 0.132 | - |
| | Emisomma3 | 0.131 | - | 0.146 | - | | Gidankambari3 | 0.116 | - | 0.13 | - |
| Negative Control | | 0.183 | | 0.12 | | Negative Control | | 0.183 | | 0.12 | |
| Positive Control | | 2.822 | | 1.137 | | Positive Control | | 2.822 | | 1.137 | |

LGA= Local Government Areas, OD= Optical Density Value, CMV= *Cucumber mosaic virus*, OkMV= *Okra mosaic virus*

Table 4.2.Cont'd.Serological detection of okra farms and distribution of *Cucumber mosaic* and O*kra mosaic* viruses concentration from some Local Government Areas of Niger State in 2019

| LGA | Location | ODV | CMV | ODV | OkMV | LGA | Location | ODV | CMV | ODV | OkMV |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Emisomma4 | 0.142 | - | 0.153 | - | | Gidan-Kambari4 | 0.126 | - | 0.138 | - |
| | Emisomma5 | 0.463 | + | 0.168 | - | | Gidan-Kambari5 | 0.106 | - | 0.132 | - |
| | Gbarifu1 | 0.146 | - | 0.15 | - | | Ibbi1 | 0.134 | - | 0.153 | - |
| | Gbarifu2 | 0.152 | - | 0.147 | - | | Ibbi2 | 0.128 | - | 0.166 | - |
| | Gbarifu3 | 0.217 | - | 0.147 | - | | Ibbi3 | 0.119 | - | 0.167 | - |
| | Gbarifu4 | 0.15 | - | 0.153 | - | | Ibbi4 | 0.148 | - | 0.183 | - |
| | Gbarifu5 | 0.165 | - | 0.163 | - | | Ibbi5 | 0.119 | - | 0.167 | - |
| | Mungorota1 | 0.116 | - | 0.187 | - | | Kawo1 | 0.169 | - | 0.167 | - |
| | Mungorota2 | 0.149 | - | 0.167 | - | | Kawo2 | 0.121 | - | 0.152 | - |
| | Mungorota3 | 0.146 | - | 0.21 | - | | Kaow3 | 0.117 | - | 0.142 | - |
| | Mungorota4 | 0.142 | - | 0.227 | - | | Kawo4 | 0.108 | - | 0.145 | - |
| | Mungorota5 | 0.176 | - | 0.149 | - | | Kawo5 | 0.12 | - | 0.188 | - |
| Mokwa | GTC1 | 0.264 | - | 0.139 | - | Paikoro | Gbadna1 | 0.21 | - | 0.135 | - |
| | GTC2 | 0.273 | - | 0.137 | - | | Gbadna2 | 0.239 | - | 0.142 | - |
| | GTC3 | 0.193 | - | 0.143 | - | | Gbadna3 | 0.219 | - | 0.118 | - |
| | GTC4 | 1.563 | + | 0.137 | - | | Gbadna4 | 0.231 | - | 0.00 | - |
| | GTC5 | 0.188 | - | 0.151 | - | | Gbadna5 | 0.15 | - | 0.124 | - |
| | Railstation1 | 0.243 | - | 0.147 | - | | Gbaita1 | 0.145 | - | 0.138 | - |
| | Railstation2 | 0.229 | - | 0.161 | - | | Gbaita2 | 0.27 | - | 0.184 | - |
| | Railstation3 | 0.238 | - | 0.147 | - | | Gbaita3 | 0.171 | - | 0.134 | - |
| | Railstation4 | 1.426 | + | 0.128 | - | | Gbaita4 | 0.336 | - | 0.127 | - |
| | Railstation5 | 0.124 | - | 0.12 | - | | Gbaita5 | 0.281 | - | 0.147 | - |
| | Waabi1 | 1.15 | + | 0.148 | - | | Jankpan1 | 0.181 | - | 0.144 | - |
| | Waabi2 | 0.213 | - | 0.151 | - | | Jankpan2 | 0.167 | - | 0.174 | - |
| | Waabi3 | 0.129 | - | 0.141 | - | | Jankpan3 | 1.249 | + | 0.14 | - |
| | Waabi4 | 0.446 | + | 0.138 | - | | Jankpan4 | 0.164 | - | 0.128 | - |
| | Waabi5 | 0.146 | - | 0.00 | - | | Jankpan5 | 0.151 | - | 0.139 | - |
| Negative Control | | 0.183 | | 0.12 | | Negative Control | | 0.183 | | 0.12 | |
| Positive Control | | 2.822 | | 1.137 | | Positive Control | | 2.822 | | 1.137 | |

LGA= Local Government Areas, OD= Optical Density Value, CMV= *Cucumber mosaic virus*, OkMV= *Okra mosaic virus*, GTC= Government Teachers College

Table4. 2. Cont'd. Serological detection of okra farms and distribution of *Cucumber mosaic* and O*kra mosaic* viruses concentration from some Local Government Areas of Niger State in 2019

| Location | ODV | CMV | ODV | OkMV | LGA | Location | ODV | CMV | ODV | OkMV |
|---|---|---|---|---|---|---|---|---|---|---|
| Wuya-Kede1 | 0.176 | - | 0.119 | - | | Jyikpe1 | 0.475 | + | 0.137 | - |
| Wuya-Kede2 | 1.854 | + | 0.135 | - | | Jyikpe2 | 1.28 | + | 0.136 | - |
| Wuya-Kede 3 | 0.56 | + | 0.152 | - | | Jyikpe3 | 1.429 | + | 0.136 | - |
| Wuya-Kede4 | 0.146 | - | 0.141 | - | | Jyikpe4 | 0.412 | + | 0.143 | - |
| Wuya-Kede5 | 0.148 | - | 0.00 | - | | Jyikpe5 | 0.122 | - | 0.138 | - |
| Negative Control | 0.183 | | 0.12 | | | Negative Control | 0.183 | | 0.12 | |
| Positive Control | 2.822 | | 1.137 | | | Positive Control | 2.822 | | 1.137 | |

OD= Optical Density Value, CMV= *Cucumber mosaic virus*, OkMV= *Okra mosaic virus*

**A** **B**

Plate II: Symptom of *Cucumber mosaic virus* disease (A) 3 weeks after inoculation compared with healthy (B) okra plants under screenhouse condition

Table 4.3. Disease incidence and severity on okra genotypes infected with *Cucumber mosaic virus* under screenhouse conditions

| | Disease incidence (%) | | | Disease severity | | |
|---|---|---|---|---|---|---|
| Genotype ID | 1 WAI | 2 WAI | 3 WAI | 3 WAI | 4 WAI | 5 WAI |
| OKR_19_01 | 100.0[a] | 100.0[a] | 100.0[a] | 2.3[a] | 2.3[a] | 2.0[a] |
| OKR_19_02 | 100.0[a] | 100.0[a] | 100.0[a] | 2.3[a] | 2.3[a] | 2.0[a] |
| OKR_19_03 | 33.3[b] | 88.9[a] | 88.9[a] | 2.0[a] | 2.0[a] | 2.0[a] |
| OKR_19_04 | 33.1[b] | 66.6[a] | 66.6[a] | 2.3[a] | 2.3[a] | 2.3[a] |
| ± SEM | 9.5 | 11.1 | 11.1 | 0.3 | 0.3 | 0.2 |

Means with dissimilar superscript within the column differ significantly at $p$=0.05 by the Least Significant Difference (LSD)

WAI = Week(s) After Inoculation

significantly different from 6.4 cm observed in the infected plants of "OKR_19_01" and "OKR_19_02". Conversely, uninoculated plants of "OKR_19_03" with a mean height of 18.9 cm were the tallest and the value obtained differed significantly from 10 cm, 11.3 cm, and 10.9 cm observed on "OKR_19_01", "OKR_19_02" and "OKR_19_04", respectively (Table 4.4).

#### 4.1.4.2 Effect of *Cucumber mosaic virus* infection on number of leaves per plant

The effect of CMV on numbers of leaves per plant varied with okra genotype (Table 4.4). Infection resulted in mosaic on leaves and leaf curling. At 2 WAI, considering the CMV-infected plants alone, "OKR_19_01" produced the highest number of leaves per plant (4 leaves/ plant) which differed significantly from a mean of 3 leaves per plant observed on other genotypes. A similar trend was observed among the healthy plants of the same genotype in which "OKR_19_01" produced the highest number of leaves per plant (6 leaves/ plant) while other genotypes produced a uniform number of leaves per plant (5 leaves/ plant).

At 4 WAI, number of leaves per plant was exactly as observed at 2 WAI among the CMV-infected plants. However, a slightly different scenario was observed with respect to uninoculated plants in which increased leaf production was observed only on "OKR_19_02" plants (7 leaves/plant), whereas number of leaves remained constant on the other genotypes.

At 6 WAI, the number of leaves increased to 7, 6 and 4 per plant on "OKR_19_01", "OKR_19_02" and "OKR_19_03", respectively for the CMV-infected plants while that of "OKR_19_04" was as reported at 4 WAI. Although there was no significant difference in number of leaves per plant between "OKR_19_01" (8 leaves/plant) and "OKR_19_02" (7

Table 4.4: Plant height and number leaves of okra genotypes infected with *Cucumber mosaic virus* under screenhouse conditions

| | Plant height (cm) | | | | | | Leaves per plant (no.) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Genotype ID | 2 WAI | Control | 4 WAI | Control | 6 WAI | Control | 2 WAI | Control | 4 WAI | Control | 6 WAI | Control |
| "OKR_19_01" | $4.0^{a}$ | $5.7^{a}$ | $5.3^{b}$ | $10.5^{a}$ | $6.4^{b}$ | $10.5^{b}$ | $4^{a}$ | $6^{a}$ | $4^{a}$ | $6^{b}$ | $7^{a}$ | $8^{a}$ |
| "OKR_19_02" | $3.5^{a}$ | $4.9^{b}$ | $5.8^{b}$ | $10.9^{a}$ | $6.4^{b}$ | $11.3^{b}$ | $3^{b}$ | $5^{b}$ | $3^{b}$ | $7^{a}$ | $6^{a}$ | $7^{a}$ |
| "OKR_19_03" | $3.6^{a}$ | $5.1^{ab}$ | $7.9^{a}$ | $12.1^{a}$ | $17.3^{a}$ | $18.9^{a}$ | $3^{b}$ | $5^{b}$ | $3^{b}$ | $5^{b}$ | $4^{b}$ | $5^{b}$ |
| "OKR_19_04" | $2.9^{a}$ | $4.9^{b}$ | $5.7^{b}$ | $7.9^{a}$ | $9.6^{b}$ | $10.9^{b}$ | $3^{b}$ | $5^{b}$ | $3^{b}$ | $5^{b}$ | $3^{b}$ | $5^{c}$ |
| ±SEM | 0.4 | 0.2 | 0.3 | 1.5 | 1.4 | 2.3 | 0.2 | 0.2 | 0.4 | 0.7 | 0.7 | 0.4 |

Means with dissimilar superscripts within the column differ significantly ($p \leq 0.05$) by the Least Significant Difference (LSD)

WAI = Weeks after inoculation

leaves/plant) among uninoculated plants, the values obtained differed significantly from a mean of 5 leaves per plant obtained from "OKR_19_03" and "OKR_19_04" (Table 4.4).

#### 4.1.4.3 Effect of *Cucumber mosaic virus* infection on number of days to flowering

The effect of CMV infection on number of days varied among the genotypes evaluated (Table 4.4). Considering the infected genotypes alone, significant differences ($p$<0.05) was observed among the genotypes with genotype OKR19_04 which flowered earlier at 28 days after planting (DAP) followed by OKR19_02 (30), OKR19_01 (32) and OKR19_03 which flowered late at 33 DAP. Among the healthy genotypes, genotype OKR19_01 and OKR19_04 flowered earlier at 29 DAP while genotype OKR19_02 and OKR19_03flowered late at 30 DAP. Significant differences ($p$<0.05) was also observed among the infected genotypes (Table 4.5)

#### 4.1.4.4 Effect of *Cucumber mosaic virus* infection on leaf length at 30 days and number of fruits per plant

The effect of CMV on leaf length varied significantly among the genotypes (Table 4.5). The infected plants produced shorter leaves compared to the healthy ones. Significant differences ($p$<0.05) were observed between leaf length of both the healthy and the infected genotypes. Leaf length of the healthy genotypes ranged from 6.2 cm (OKR19_03) to 8.7 cm (OKR19_03) while infected genotypes exhibited leaf length of 5.0 cm (OKR19_04) to 6.2 cm (OKR19_01). Effect of CMV on number of fruits for both the healthy and infected genotypes was not significant ($p$<0.05). Furthermore, both the healthy and infected plants produced an average of 1 fruit each for all the genotypes evaluated in this study (Table 4.5).

Table 4.5: Morphological characters of okra genotypes infected with *Cucumber mosaic virus* under screenhouse conditions

| Genotypes I D | Number of days to flowering | | Leaf length at 30 day (cm) | | Number of fruits per plant | |
|---|---|---|---|---|---|---|
| | Infected | Healthy | Infected | Healthy | Infected | Healthy |
| OKR19_01 | $32^{ab}$ | $29^{bc}$ | $6.2^{a}$ | $8.1^{b}$ | $1^{a}$ | $1^{a}$ |
| OKR19_02 | $30^{bc}$ | $30^{ab}$ | $7.1^{a}$ | $8.6^{ab}$ | $1^{a}$ | $1^{a}$ |
| OKR19_03 | $33^{a}$ | $30^{a}$ | $6.3^{ab}$ | $8.7^{a}$ | $1^{a}$ | $1^{a}$ |
| OKR19_04 | $29^{c}$ | $29^{c}$ | $5.0^{b}$ | $6.2^{c}$ | 1a | $1^{a}$ |
| ±SEM | 0.8 | 0.2 | 0.3 | 0.2 | 0.0 | 0.2 |

Means with dissimilar superscripts within the column differ significantly ($P \leq 0.05$) by the Least Significant Difference (LSD)
WAI= Week(s) After Inoculation

#### 4.1.4.5 Effect of *Cucumber mosaic virus* infection on fruit length per plants

The effect of CMV on fruit length varied significantly among the genotypes evaluated (Table 4.6). Considering the CMV-infected plants, the fruits of "OKR_19_01" genotype were the longest (5.3 cm). The plants "OKR_19_03" and "OKR_19_04" produced similar fruit length (1.9 cm) which was statistically comparable to the fruit length of "OKR_19_02" (1.2 cm). Similar to the CMV-infected plants, the fruits from uninoculated "OKR_19_01" plants were the longest (8.8 cm) whereas there were significant differences in fruit of the other genotypes. Meanwhile, the fruits of uninoculated "OKR_19_04" plants (4.0 cm) were longer than those of "OKR_19_02" (1.7 cm) and "OKR_19_03" (3.4 cm) (Table 4.6).

#### 4.1.4.6 Effect of *Cucumber mosaic virus* infection on fruit weight per plants

Infection of okra plant reduced fruit weight significantly, with values ranging between 1.5 g in "OKR_19_04" and 4.3 g in "OKR_19_01" per plant (Table 4.6). However, the highest fruit weight obtained from "OKR_19_01" did not differ significantly from a mean of 3.9g observed on "OKR_19_03" plants. On the other hand, a mean fruit weight of 2.8 g came from "OKR_19_02" plants. Uninoculated plants produced fruit weights that varied significantly between 1.2 g (OKR_19_04) and 6.1 g (OKR_19_01). The mean height of "OKR_19_02" (5.3 g/plant) was also high and statistically comparable to that of "OKR_19_01", whereas the mean fruit weight of uninoculated "OKR_19_03" was 4.1 g per plant (Table 4.6).

#### 4.1.4.7 Effect of *Cucumber mosaic virus* infection on fruit diameter per plants

Similar to the results of other yield parameters, CMV disease induced significant differences in fruit diameter among the okra genotypes (Table 4.6). The genotype "OKR_19_01" produced fruits with the widest diameter (2.5 cm) but the value obtained did not differ statistically similar from a mean of 1.8 cm and 1.9 cm observed on "OKR_19_03" and "OKR_19_04", respectively. The lowest fruit diameter (0.3 cm) came from "OKR_19_02" plants. Uninoculated plants produced fruit diameters spanning between 0.9 cm in "OKR_19_02" and 3.4 cm in "OKR_19_01". Moreover, the mean fruit diameter of "OKR_19_04" plants (2.9 cm) was higher than "OKR_19_03" plant (2.3 cm) but the difference was not significant.

Table 4.6: Fruit length, weight and diameter of okra genotypes infected with *Cucumber mosaic virus* under screenhouse conditions

| | Fruit length (cm) | | Fruit weight per plant (g) | | Fruit diameter (cm) | |
|---|---|---|---|---|---|---|
| Genotype ID | Infected | Control | Infected | Control | Infected | Control |
| "OKR_19_01" | 5.3[a] | 8.8[a] | 4.3[a] | 6.1[a] | 2.5[a] | 3.4[a] |
| "OKR_19_02" | 1.2[b] | 1.7[b] | 2.8[ab] | 5.3[a] | 0.3[b] | 0.9[b] |
| "OKR_19_03" | 1.9[b] | 3.4[b] | 3.9[a] | 4.1[ab] | 1.8[a] | 2.3[ab] |
| "OKR_19_04" | 1.9[b] | 4.0[b] | 1.5[b] | 1.2[b] | 1.9[a] | 2.9[ab] |
| ±SEM | 0.8 | 1.0 | 0.5 | 0.9 | 0.3 | 0.7 |

Means with dissimilar superscripts within the column differ significantly ($p \leq 0.05$) by the Least Significant Difference (LSD)

WAI= Weeks(s) After Inoculation

## 4.2 Discussion

*Cucumber mosaic virus* disease was more prevalent than OkMV in the study area probably due to availability of several aphid vectors and ease of transmission within 5 to 10 seconds (Dragich *et al.,* 2014). Additionally, the higher incidence of CMV disease in farmers' field revealed its s spatial distribution and pathogenicity on okra plants. Thus, Nouri *et al*. (2014) earlier reported that CMV disease parasitizes several crops of economic importance and weed species. Also, the wide distribution of CMV was possibly a consequence of poor agronomic practices including cultivation of seeds from the previous harvest. Studies have shown that CMV is transmitted through infected seeds, another agent responsible for wide dissemination of several plant viruses. Continuous seed recycling for new field establishment encourages inoculum build-up which exacerbates disease incidence. Incidence of OkMV disease was relatively low probably because it is mainly transmitted by insects (particularly flea beetles: *Podagrica* spp). Although both CMV and OkMV diseases are transmitted non-persistently, the virus-infected plants serve as sources of primary inoculum for further spread within the field. Moreover, the continuous cropping of okra alongside other crops like pepper, cucumber and cowpea might have greatly aggravated the spread of these viruses. Therefore, the relatively low OkMV incidence could also be attributed to high level of intercropping of okra with non-host plants.

The data from field survey showed that CMV and OkMV were not present in same location. This indicates that majority of the symptomatic plants encountered during the survey were induced by other pathogens or due to lack of nutrients in the soil. This corroborates the findings of Salaudeen *et al.* (2018) who reported that symptoms on plants are not only

induced by viruses alone but also due to other pathogens (fungus and bacteria). Some leaves without symptoms tested positive for viruses. This indicates that the absence of visual symptoms on leaves is not enough to conclude that the crop plant is immune to virus infection. Thus, the detection through laboratory diagnosis serves as more sensitive and conclusive method of affirming the health and resistance of a plant. Complete infection of "OKR_19_01" and "OKR_19_02" plants as early as 1 WAI revealed their vulnerability to CMV disease. This is in tandem with Paudel and Sanfaçon (2018) who documented that following virus entry, a series of physiological interactions is activated in the host plant. The ability of host plant to restrict virus replication and movement is very critical and determines symptoms expression in the host plants. However, the disease severity data indicated relatively low level of pathogenicity of CMV on the evaluated okra cultivars. Despite high incidence observed among the okra cultivars, disease severity was relatively mild. Thus, it could be argued that the reaction of host plant defense mechanisms was effective against virus replication and intercellular movement within the host plant.

Plant height and number of leaves per plant were significantly lower in the CMV-infected plants compared to the healthy plants. This was due to deleterious effect of CMV disease which altered hosts' ability to absorb nutrients and moisture from the soil for proper functioning and growth potentials. Studies have shown that viruses can influence plant defense chemistry, including the constitutive and induced expression of phytohormones that mediate defense responses to herbivory and infection1(Mauck *et al*., 2012), and alter plant nutritional quality by diverting resources towards virus replication and shifting the allocation of nutrients among host tissues (Mauck *et al*., 2014). Consequently, these viruses induced

changes in host-plant architecture, size, leaf morphology and yield characters. Although none of the CMV-infected plants attained their full potential in terms of fruit length, fruit weight per plant and fruit diameter, when compared with uninoculated plants, “OKR_19_01” gave the highest values of these parameters among the virus-infected plants. This result implies that it was the most tolerant to CMV disease. This agrees with Paudel and Sanfaçon (2018) who stated that tolerant plants mitigate the impact of virus infection irrespective of the pathogen load. Therefore, despite the accumulation of a significant virus titre, the plant growth, yield or reproduction attributes are only minimally affected and visible symptoms are either absent or mild.

# CHAPTER FIVE

# 5.0 CONCLUSION AND RECOMMENDATIONS

## 5.1 Conclusion

This study has revealed the occurrence of CMV and OkMV in the study area. The study also revealed the pathogenic effect of *Cucumber mosaic virus* on plants' growth and yield characteristics of the evaluated okra genotypes. Although100 % incidence of CMV disease was found on "OKR_19_01" plants as early as 1 WAI, it eventually gave the greatest yield performed among the okra genotypes evaluated.

## 5.3 Recommendations

Okra genotype "OKR_19_01" is recommended to farmers in CMV-endemic locations for better productivity. However, further extensive survey is needed so as to reveal virus diversity in okra farmlands. In the meantime, the okra genotypes "OKR_19_01" could be adopted in CMV-endemic areas. Plant breeders could also cross this genotype with CMV-resistant genotype for improved resistance and yield.

## REFERENCES


Abdel-kadel, A. A., Shaaban**,** S. M. & Abd El-Fathah., M. S. (2010). Effect of irrigation levels and organic compost on okra plant (*Abelmoschus esculentus*) grown in sandy calcerouse soil, *Agricultural and Biological Journal of North America,* 1, 225-231.

Abidi, A. B., Priya, S., Varun C., Brahm, K. T., Shubhendra, S. C., Sobita, S. & Bilalc, S. (2014). An overview on Okra (*Abelmoschus esculentus*) and its importance as a nutritive vegetable in the World. Department of Biochemistry & Biochemical Engineering, *International Journal of Pharmacy and Biological Sciences*, 2230-7605.

Adeboye, M. K., Bala, A., Osunde, A. O., Ozuma, A. O., Odofin, J. A. & Lawal., B. A (2011). Assessment of soil quality using soil organic carbon and total nitrogen and microbial properties in tropical agro-ecosystem. *Agricultural science*, 2, 34 - 40

Adetuyi, F. O., Osagie, A. U. & Adekunle A. T. (2008). Effect of Postharvest Storage Techniques on the Nutritional Properties of Benin Indigenous Okra *Abelmoschus esculentus* (L) Moench. *Pakistan Journal Nutrition*, 7, 652-657.

Adilakshmi, A., Korat, D. M. & Vaishaw P. R. (2010). Effect of organic and inorganic fertilizer on insect pest infecting okra. *Karnataka Journal of Agricultural Sciences*, 21, 287-289.

Akande, M. O., Oluwatoyinbo, F. I., Makinde, E. A., Adepoju, A. S. & Adepoju, I. S. (2010). Response of okra to organic and inorganic fertilization. *Nature Science*. 8, 261-266

Akintoye, H. A., Adebayo, A. G. & Aina, O. O. (2011). Growth and yield responses of okra intercropped with live mulches. *Asian Journal of Agriculture*, 5, 146-153.

Aladele, S. E., Ariyo, O. J. & Lapena, R. (2008). Genetic relationship among West African okra (*Abelmoschus caillei*) and Asian genotypes (*Abelmoschus esculentus*) using RAPD. *African Journal of Biotechnology,* 7, 1426-1431.

Alegbejo, M. D. (2015). Virus and virus-like Diseases of Crops in Nigeria. Ahmadu Bello University Press Limited, Zaria, Kaduna State, Nigeria. 272pp

Arapitsas, P. (2008). Identification and qualification of polyphenolic compound from okra seed and skins. *Food chemistry*, 110, 1041-1045.

Ariyo, O. J. & Lapene, R. (2008). Genetic relationship among West Africa okra (*Abelmoschus callei*) and asian genotype (*Abelmoschus esculentus*) using RAPD. *African Journal of Bilogical Technology*, 7, 1426-1045.

Asare, B. E., Agyarko. F., Taah, K. J., Asare, A., Kwame, A.F. & Sarfo, J. (2018). Phenotypic and serological screening of okra genotypes against *Okra mosaic virus* infection under field conditions. *Ruforum Working Document Series,* 14 (1), 571-580.

Asare, B. E., Van der Puije, G. C., Taah, K. J., Abole, E. A. & Baidoo, A. (2014). Prevalence of *okra mosaic* and *leaf curl diseases* and *Podagrica* spp. *International Journal of Current Research and Academic Review,* 2(6), 260– 271.

Askira, A. B. (2012). A survey on the incidence of *okra leaf curl virus* on okra in Lake Alau Area of Borno State, Nigeria. *International Journal of Agriculture,* 1-6.

Benchasri, S. (2012). Screening for *yellow vein mosaic virus* resistance and yield loss of okra under field conditions in Southern Thailand. *Journal of Animal and Plant Science,* 12, 1676-1686

Bushra, A., Geetesh, B., Mehar, F., Mohammed, U. & Naqvi, Q. A. (2012). Studies on Molecular Detection of *Cucumber mosaic virus* and Its Anatomical and Biochemical Changes in *Daucus carota* L. 1,187-192.

Chaudhary, A., Khan, M. A. & Bilal, Y. (2016). Management of *Okra yellow mosaic virus* and its vectors through plant extracts. *International Journal of Plant Pathology and Microbiology,* 8: 393-396

Dada, O. A. & Fayinminnu O. O. (2010). Period of weed control in okra (*Abelmoschus esculentus*) as influenced by varying rates of cattle dung and weed regimes. *Notulae Botanicae Hortucultuer Agrobotanici Cluj- Napoc*a, 38, 149-151.

Dilruba S., Hasanuzzaman M., Karim, R. & Nahar, K. (2009). Yield response of okra to different sowing time and application of growth hormones. *Journal Horticultural Science and Ornamental Plants,* 1, 10-14.

Dragich, M., Melzer, M. & Nelson, S. (2014). *Cucumber mosaic virus* in Hawai'i. *Plant Disease,* 1 – 10.

Fajinmi, A. A. & Fajinmi, O. B. (2010). Incidence of *Okra mosaic virus* at different growth stages of okra plants *Abelmuscus esculentus* L. Under Tropical Condition. *Journal of General and Molecular Virology,* 2 (1), 028-031

Fajinmi, A. A. & Odebode, C. A. (2010). Evaluation of maize/pepper intercropping model in the management of *Pepper veinal mottle virus*, genus *Potyvirus*, family *Potyviridae* on cultivated pepper (*Capsicum annum* L.) in Nigeria. *Archives of Phytopathology and Plant Protection,* 43 (15), 1524 - 1533

FAO (Food and Agriculture Organization), (2017). Food and Agriculture organization of the United Nations, Retrieved on 18th April, 2018 from http://faostat.fao.org/faostat/

FAO (Food and Agriculture Organization), (2019). Food and Agriculture organization of the United Nations, Retrieved on 30th August, 2021 from http://faostat.fao.org/faostat/

Haruna, I. M. & Jabil, I. J. (2017). Survey on the effect of *Okra mosaic virus* and *leaf curl virus* on yield of okra in Maiduguri, Barno State, Nigeria. *International Journal of Sciences and Applied Research,* 2(3), 2504-2508

Handiseni, M., Sibiya, J., Ogunlela, V. & Koomen, T. (2010). Evaluation of non-chemical methods of sterilization in Paprika (*Capsicum annum*L.) seedling production in small holder farming in Zimbabwe. *Agricultura Tropica EtSubtropica,* 43 (2), 97-108.

Iyagba, A.G., Onuegbu, B.A. & Ibe, A. E. (2013). Growth and yield response of okra (*Abelmoschus esulentus* (L.) Moench) to NPK fertilizer rates and weed interference in Southeastern Nigeria. *International Research Journal of Agriculture Science and Soil Science,* 3(9), 328-335

Kator, P. E., Ogbinaka, E. & Omamode, C.O. (2015). A comparative analysis of the growth performance of okra (*Abelmuschus esculentus*) in different soil media. *Global Advance Research of Agricultural Sciences,* 4 (10), 649-656

Kumar, R., Patil, M. B. & Paschoir, M. S. (2009). Evaluation of *Abelmoschus* mucilage as suspending agent in paracetamol suspension. *International Journal of Pharmacy*, 1, 658-668.

Kumar, S., Dagnoko, S., Haougui, A., Ratnadass, A., Pasternak, D. & Kouame, C. (2010). Okra (*Abelmoschus* spp.) in West and Central Africa: potential and progress on its improvement. *African Journal of Agricultural Research*, 5: 3590-3598.

Kumar, R., Solankey, S. S., Shirin, A., Varma, R. B. & Kumar, S. (2014). Seasonal response of okra (*Abelmoschus esculentus* L.Moench) genotypes to *Okra yellow vein mosaic virus* incidence. *African Journal of Biotechnology*, 13(12), 1336-1346

Mauck, K., Bosque-Perez, N. A., Eigenbrode, S. D., De Moraes, C. M. & Mescher, M. C. (2012). Transmission mechanisms shape pathogen effects on host-vector interactions: evidence from plant viruses. *Functional Ecology*, 26 (5):1162 – 1175.

Mauck, K. E., De Moraes, C. M. & Mescher, M. C. (2014). Biochemical and physiological mechanisms underlying effects of *Cucumber mosaic virus* on host-plant traits that mediate transmission by aphid vectors. *Plant, Cell and Environment,* 37, 1427 –1439

Mark, D., Micheal, M. & Scot, N. (2014). Department of plant protection and Environmental Protection Sciences, Collage of Tropical Agriculture and Human Resources, University of Manda, Hawai`i.

Mathew, C. O., Mosses, E. O., Raymond, O. A. & Rosemary, N. I. (2016). Ethnobotany and collection of West African Okra {*Abelmoschus caillei* (A. Chev.) Stevels}Germplasm in some communities in Edo and Delta States, Southern Nigeria. *Borneo Journal Resource Science and Technology,* 6(1): 25-36

Nouri, S., Arevalo, R., Falk, B. W. & Groves, R. L. (2014). Genetic *structure and molecular variability of Cucumber mosaic virus isolates in the United States. PLoS ONE,* 9(5): e96582. doi:10.1371/journal.pone.0096582

Ngbede, S. O., Ibikwe. H. N, Okpara. S. C., Omyegbule, U. N. & Adejumo, L. (2014). An overview of Okra Production, Processing, Marketing, Utilization and Constraints in Ayaragu in Ivo Local Government Area of Ebonyi state, Nigeria. *Greener Journal of Agricultural Science*, 4 (4), 136-143.

Paudel, D. B. & Sanfaçon, H. (2018). Exploring the diversity of mechanisms associated with plant tolerance to virus infection. *Frontiers in Plant Science*, 9:1575. doi: 10.3389/fpls.2018.01575

Saifullah, M. & Rabbani, M. G. (2009). Evaluation and characterization of okra (*Abelmoschus esculentus* L. Moench) genotypes. *Journal of Agriculture,* 7, 92-99.

Salaudeen, M. T., Gana, A. S., Bello, L. Y., Daudu, O. A. J. & Oyewale, R. O. (2018). Survailence of Maize Lethal Necrosis Diseases in Nigeria. *Production Agriculture and Technology,* 14(2): 68-77.

Tripathi, K. K., Govila, O. P., Warrier, R. & Shuja, V. (2011). Biology of *Abelmoschus esculentus* {L.} Okra series of Crop Specific Biology Documents. Department of Biotechnology, Ministry of Science and Technology and Ministry of Environmentand Forestry, Government of India, 1-10.

Varmudy, V. (2011).Marking survey need to boost okra export. Department of economics, Vivekananda Collage, Putur, Karntaka, India.

Yusuf, S. M., Kashina, B. D., Alegbejo, M. D. & Bamwo, O. O (2018). Survey of Viruses Associated With Field-Grown Sweet Melon (*Cucumis Melo* L.) in Gombe State. North East Nigeria. *Nigerian Journal of Plant Protection*, 32: 94-101

Zitter, T. A. & Murphy, J.F (2009).*Cucumber Mosaic Virus, the plant health instructor.* DOI: 10.1094/PHI-1-2009-1518-01.

**Appendix 1**

**Questionnaire**

**Survey of Okra Viruses in selected Local Government Areas of Niger State in 2019 cropping season**

Date: _____ season: _____

LGA: _____ Name of village: _____

Latitude: _____ Longitude: _____

Elevation: _____

Gender of farmer: Male: ( ) Female: ( )

Highest educational background: (A) Primary (B) Secondary (C) Tertiary (D) Non-formal

Okra farming experience (years): _____ Size of the okra farm: _____

Purpose of cultivation: (A) Consumption (B) Sale (C) Consumption and sale

Source of Seeds: (A) ADP (B) Research Institutes (C) Agro-Shops (D) friends (E) previous harvests

(F) Market

What variety of okra do you plant? _____

Why are you interested in that variety? _____

Which seasons of the year do you plant okra? (A) Dry season (B) Wet season(C) Dry and Wet season

Do you practice intercropping? (A) Yes (B) No

If yes, with what crop(s)? _____

Do you practice crop rotation? (A) Yes (B) No

If yes, with what crop do you rotate okra? _____

What is the length of rotation? _____

Do you experience insects on the okra plants? (A) Yes (B) No

What growth stage do you notice insects? _____

Do you use insecticide? (A) Yes (B) No

If yes, what type? _____

Do you use fertilizer? (A) Yes (B) No

If yes, what type? _____

Do you use herbicide? (A) Yes (B) No

If yes, what type? _____

What are the types of crops surrounding the farm? _____

Field symptom severity score (score 1-5) for okra leaves samples

| **LGA/District** | **Village/ Community** | **Leave Sample** | **Severity Score** | | | | |
|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 4 | 5 |
| | | | | | | | |

Average severity score _____ % of okra leaves sample

Key; 1.No obvious symptoms, 2.Symptoms on 0 – 24 % of leaves, 3.Symptoms on 25 – 50 % of leaves4.Symptoms on 51 – 74 % of leaves, 4. Symptoms on 75 Symptoms on 0 – 24% of leaves.