OCCURRENCE, DISTRIBUTION AND ALTERNATIVE HOSTS OF JATROPHA MOSAIC VIRUS IN SOME NORTHWESTERN STATES OF NIGERIA

BY

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NOVEMBER, 2018

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BY

Agaat Charles CHINDO, B.Agric (A.B.U.) 2010

P16AGCP8033

A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER DEGREE IN

CROP PROTECTION

DEPARTMENT OF CROP PROTECTION,

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AHMADU BELLO UNIVERSITY,

ZARIA, NIGERIA

NOVEMBER, 2018

DECLARATION

I declare that the work in this Dissertation titled **"Occurrence, Distribution and Alternative Hosts of Jatropha Mosaic Virus in some North Western States of Nigeria"** has been carried out by me in the Department of Crop Protection, Ahmadu Bello University, Zaria. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation has been presented for another degree or diploma at any other institution.

CHINDO, Agaat Charles _____ Signature _____ Date

CERTIFICATION

This Dissertation titled, **"OCCURRENCE, DISTRIBUTION AND ALTERNATIVE HOSTS OF JATROPHA MOSAIC VIRUS IN SOME NORTH WESTERN STATES OF NIGERIA"** by Agaat Charles CHINDO meets the regulations governing the award of the degree of Master of Science in Crop Protection of the Ahmadu Bello University, Zaria and is approved for its contribution to scientific knowledge and literary presentation.

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DEDICATION

This work is dedicated to God Almighty, the creator of heaven and earth and the owner of my life for blessing me with the gift of life and my beloved parents, Professor and Mrs. P.S. Chindo.

ACKNOWLEDGEMENTS

All gratitude to God Almighty for keeping me alive and guiding me through my years of study. My appreciation largely goes to my supervisor Professor B.D. Kashina for his guidance through difficult times and continuous belief in me and to the rest of my supervisory team Professors O.O. Banwo, M.D. Alegbejo for all your constructive criticisms. I am also grateful to Professor A.B. Zarafi, Professor L.J. Bamaiyi, Messers A.J. Kwanashie, I.J. Ajene, M.O. Jibrin and A.O. Abdullahi and all members of staff of Crop Protection Department for their invaluable inputs and criticisms. Much gratitude goes to the Biotechnology Research Program of Institute for Agricultural Research, Ahmadu Bello University and Department of Crop Protection for providing the facilities, favourable environment and necessary funding, which led to the completion of this work.

Many thanks to Mr Suleiman Umar and the entire staff of the Biotechnology Research laboratory, Institute for Agricultural Research, Samaru, Zaria who were with me throughout my laboratory experiments. The technical support of staff of the Virology Laboratory at the Department of Crop Protection, Faculty of Agriculture, Ahmadu Bello University is duly acknowledged.

The support and love of the entire staff and students of Department of Crop Protection, Chindo's family, Elah Oganya Lucy is also greatly acknowledged.

ABSTRACT

Field survey was conducted in 2015 between August and September in three Local Government Areas of Kaduna, Katsina and Kano States of Nigeria to determine the incidence, distribution and alternative hosts of Jatropha Mosaic Virus on Physic nut (*Jatropha curcas* L). A total of twenty seven (27) *Jatropha curcas* farms were surveyed in three States (Kaduna, Katsina and Kano), nine in each State. Sampling was done in three demarcated 12 × 12 m^2 plot in each of the selected fields per Local Government Area. *Jatropha curcas* and other plant species showing virus like symptoms which included mosaic, leaf curl, mottling, stunting, chlorosis, yellowing, and necrotic symptoms as well as asymptomatic plants were sampled. A total of twenty eight weed samples were randomly collected from the three States. Analysis was carried out for the detection of viral components (DNA-A and B) and sub-viral components (DNA-β and 1) of *Jatropha mosaic virus* by Polymerase Chain Reaction (PCR). *Jatropha mosaic virus* disease was present in all farm locations and the incidences recorded. The highest disease incidence in Katsina State (95 %) was recorded at Walin-Hausa Foundation farm (Tsanni) in Batagarawa LGA, and the lowest (43.75%) in Katarda of Batagarawa LGA. In Kaduna State, the National Research Institute for Chemical Technology (NARICT) *Jatropha curcas* field had the highest incidence (83.61%), with the lowest (42.86%) at Gwaraji in Igabi LGA. The highest disease incidence (88%) recorded in Kano State was at Marmara in Tudun-Wada LGA, and lowest (2%) in the Institute for Agricultural Research (I.A.R) sub-station *Jatropha curcas* farm at Minjibir LGA. The viral components DNA-A and B were not detected in all the fields across the three States, as well as the sub-viral component DNA-1 while the sub-viral component DNA-β was detected only on some samples from NARICT and I.A.R *Jatropha*

curcas fields both in Sabon-Gari Local Government Area of Kaduna State. The helper virus component (DNA-A) was not detected. None of the plant species samples tested positive to any of the viral and sub-viral components of interest in this study from the three States. This is the first report of a satellite virus DNA-β of JMV in Kaduna State of Nigeria. In view of the importance of *Jatropha curcas* L. in Nigeria, threats such as *Jatropha mosaic virus* attack to its production should be considered seriously and prevention as well as management strategies be pursued vigorously.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Jatropha curcas L.often referred to as 'Jatropha' is one of the prospective oil-yielding plants with vast industrial potential as bio-diesel (Jayanna, 2006). The seeds can be pressed into bio-oil that has good characteristics for direct combustion in compressed ignition engines or for the production of bio-diesel (Putten *et al*., 2010). The bio-oil can also be a raw material for soap making. The pressed residue of the seeds (pressed cake) is a good fertilizer and can also be used for bio-gas production. *Jatropha curcas* grows under subtropical conditions and can withstand conditions of severe drought and low soil fertility. It is capable of growing in marginal soil, and it can help to reclaim problematic lands. It is not a forage crop, hence, it plays an important role in deterring cattle as the leaves are not palatable, and thereby, protecting other valuable food or cash crops when used in fencing (Putten *et al*., 2010). Current interest by farmers and non-governmental organizations (NGOs) in *Jatropha curcas* is mainly due to its potential as an energy crop.

Jatropha curcas grows in tropical and sub-tropical regions, with cultivation limits at 30ºN and 35ºS (FACT, 2007). It also grows in lower altitudes of 0-500 meters above sea level. It is not sensitive to day length (flowering is independent of latitude) and may flower at any time of the year (Heller, 1996). It is a succulent shrub that sheds its leaves during the dry season, with deep roots that make it well suited to semi-arid conditions. While Jatropha can survive with as little as 250 to 300 mm of annual rainfall, at least 600 mm is needed for flowering and fruit setting (FACT, 2007). The optimum rainfall for seed production is

considered between 1000 and 1500 mm (FACT, 2007), which corresponds to sub-humid ecology. Higher precipitation is likely to cause fungal attack and restrict root growth in all but the most free-draining soils (cited by Achten, 2008).*Jatropha curcas* is not found in the more humid parts of its area of origin, Central America and Mexico. Rainfall induces flowering and, in areas of unimodal rainfall, flowering is continuous throughout most of the year (FACT 2007). Optimum temperatures are between 20˚C and 28˚C. Very high temperatures can depress yields (Gour, 2006). *Jatropha curcas* is intolerant of frost. The plant is well adapted to conditions of high light intensity (Baumgaart, 2007) and is unsuited to growing in shade.

Insect pests and diseases pose a significant threat to *Jatropha curcas*. Observations of free-standing older trees would appear to confirm this, but incidence of insect pests and diseases is widely reported under monoculture plantation, and may be of economic significance. The *Jatropha curcas* plants suffer from several diseases, which include damping off, root rot, leaf spots, frog-eye leaf spot, powdery mildew and *Jatropha mosaic virus* disease. Observed diseases, such as collar rot, leaf spots, root rot and damping-off, may be controlled with a combination of cultural techniques (for example, avoiding waterlogged conditions) and fungicides (Achten, 2008). *Jatropha multifida* is a known host of *African cassava mosaic virus* as well as a possible source of transmission of the cassava super-elongation disease caused by *Sphaceloma manihoticola* (Achten, 2008).

The family *Geminiviridae* comprises of a group of plant infecting circular ssDNA viruses that severely constrain agricultural production throughout the World, and a particular serious threat to food security in sub-Saharan Africa. Worldwide, they are responsible for a

considerable amount of economic damage to many crops such as cassava, sweet potato, tomatoes, cowpea, okra, cotton, grain legumes and now *Jatropha curcas* (Fauquet *et al*., 2008).

Geminiviruses are distinct in having circular, single-stranded DNA (ssDNA) genomes that are encapsidated within twinned icosahedral virions. Displaying substantial diversity in terms of their primary nucleotide sequences, genome structures, host ranges and insect vectors, the family *Geminiviridae* has been divided into seven different genera. Besides the Begomoviruses, these include the genera *Mastrevirus, Curtovirus,* and *Topocuvirus* (Fauquet *et al*., 2008) and three additional genera Becurtovirus, Eragrovirus and Turncurtovirus (Varsani *et al*., 2014).

The genus *Begomovirus* with over one hundred and ninety two recognized species, contains more species than all the other geminivirus genera combined (Fauquet *et al*., 2008; Brown *et al*., 2011). Whereas begomoviruses are generally considered to be either monopartite (one ssDNA component) or bipartite (two circular ssDNA components called DNA-A and DNA-B), many apparently monopartite begomoviruses are associated with additional subviral ssDNA satellite components, called alpha- (DNA-1/α) or betasatellites (DNA-β) (Marie *et al*., 2012). Geminiviruses are often associated with sub-viral agents called DNA satellites that require proteins encoded by the helper virus for their replication, movement and encapsidation. Hitherto, most of the single-stranded DNA satellites reported to be associated with members of the family Geminiviridae have been associated with monopartite begomoviruses. These satellite molecules completely lack sequence identity to their helper viruses and depend on the helper virus for all or some of the following functions: replication, movement, encapsidation and transmission. Satellite molecules were initially

reported to be associated with RNA viruses; these satellites are very well characterized (Simon *et al*., 2004). In the last decade, more than 500 satellite sequences associated with begomoviruses (family Geminiviridae) have been isolated from a diverse range of cultivated crops and weeds (Briddon and Stanley, 2006; Briddon *et al*., 2008).

Alphasatellites are 1.3 kb nanovirus-like components that, in some cases, suppress viral disease symptoms. Although alphasatellites encode a replication associated protein, they depend on the helper virus encoded proteins for movement and encapsidation (Briddon and Stanley, 2006; Nawaz-ul-Rehman and Fauquet, 2009). Betasatellites are a diverse set of symptom-enhancing, single-stranded DNA (ssDNA) molecules that are 1.3 kb in size and only associated with monopartite begomoviruses from the Old World (OW), namely Asia and Africa (Briddon *et al*., 2001, 2003, 2008). Most recently, betasatellites have been found associated with a few bipartite begomoviruses (Rouhibakhsh and Malathi, 2005).

Cook in 1931 described the occurrence of Jatropha mosaic disease for the first time in Puerto Rico (USA). Jatropha mosaic virus was first reported on *Jatropha gossypiifolia* (Bird, 1957) as a euphorbiaceous weed prevalent throughout the West Indies. Several viruses have been found to affect *Jatropha curcas*. Kashina *et al*. (2013) reported a complete nucleotide sequence of a begomovirus (Jatropha Mosaic Nigerian Virus) naturally infecting *Jatropha curcas* in Nigeria which is a threat to the realization of the full potential of the crop. The symptoms observed were severe mosaic, mottling and blistering of leaves. Pair-wise comparisons of DNA-A sequences showed that *Jatropha mosaic virus* had maximum nucleotide sequence identity (72 %) with a strain of *Tomato yellow leaf curl virus*. Ramkat *et al*. (2011) reported the occurrence of cassava geminiviruses on *Jatropha curcas* grown in

Kenya. Raj *et al*. (2008a) reported the incidence of *Cucumber mosaic virus* on *Jatropha curcas* in India. Snehi *et al*. (2008) identified a new begomovirus on J. *gossypifolia* in India.

1.2 Justification of the study

The crude oil crisis of the 1970s and the subsequent shortages of petrol-fuels in the world market have brought to light the limitations of world oil resources (Grimm, 1996; Heller, 1996; Henning, 2000; Pratt *et al*., 2002). Currently, the world is faced with critical fuel shortages accompanied with high prices, as well as, the global warming issue. This has prompted government, scientists and non-governmental organizations (NGOs) to search for alternative sources of energy, which are renewable and environmentally safe. In this regard, renewable vegetable fuels have assumed top priority. Special interest has been shown in the cultivation of the tropical physic nut (*Jatropha curcas* L., Euphorbiaceae) for oil extraction (Grimm 1996; Heller 1996).Worldwide, Jatropha is gaining more prominence considering its potential as an energy and biofuel crop. Although much of this potential has not been harnessed fully in Nigeria, elsewhere it has been demonstrated that the crop can help solve many problems ranging from land reclamation, bio-diesel production and protection of valuable crops such as cash or food crops from cattle (Jayanna, 2006). The potential yield, quality and value of the croparelowered by pest attack reducing the quality of seeds produced, thereby, affecting its potential of fulfilling the promise of an energy crop. Recently, virus diseases have posed a great threat to the crop's potential due to their insidious nature, which usually results in the development of epidemics, if not managed properly.

Most of the plant viruses have weeds or other alternative natural hosts that provide a reservoir of viruses from which the economically important crops may become infected (Neeraj and Zaidi, 2008; Mathews and Dodds, 2008). Weeds, ornamental plants and wild plants near crop fieldsare infected with the viruses of cultivable plant species (Sivalingam and Varma, 2007) and play a key role in the development of virus disease epidemics, because the bio trophic nature of plant viruses obviously requires the alternate hosts to continue association of virus-host-vectors association for the sustainability of the viral pathogens in nature in the absence of original crop host. All these make the virus disease epidemiology more complex and hazardous as compared to other diseases (Maramorosch and Harris, 1981). Identification of initial source of infection is important for studying the epidemiological aspects of plant viruses and also to formulate control measures for the viral diseases (Thresh, 1982).

The expected benefit of the proposed research is that knowledge about the existence, distribution and alternative hosts of the virus in Nigeria will aid the development of effective management strategies to enhance the growth of jatropha and its success as a potential energy crop and a renewable energy source.

1.3 Objectives of the study

This research was undertaken to fulfill the following objectives:

1. To determine the incidence and distribution of *Jatropha mosaic virus* (JMV) in some Northwestern States of Nigeria.
2. To identify the alternative hosts of the virus.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 *Jatropha curcas* Classification

Jatropha curcas belongs to the Kingdom: Plantae; subkingdom: Tracheobionta; super division: Spermatophyta; division: Magnoliophyta; class: Magnoliopsida; subclass: Rosidae; order: Malpighiales/Euphorbaiales; family: Euphorbiaceae; subfamily: Crotonoideae; tribe: Jatropheae; Genus: Jatropha; Species: *curcas.* Jatropha is a morphologically diverse genus comprising of 160–175 species of trees, shrubs, rhizomatous sub-shrubs and herbs distributed mainly in the seasonally dry tropics (Dehgan, 1984). The genus *Jatropha* has many species such as *Jatropha curcas, Jatropha cuneata, Jatropha gossypiifolia, Jatropha integerrima, Jatropha multifida, Jatropha podagrica, Jatropha acanthophylla, Jatropha bullockii, Jatropha cathartica, Jatropha chamelensis, Jatropha costaricensis, Jatropha dioica, Jatropha elliptica, Jatropha excisa, Jatropha macrorhiza, Jatropha nudicaulis, Jatropha unicostata* (www.aumkiipure.com; www.desert_tropicals.com).

Curcas is the common name for physic nut in India (Correll and Correll, 1982). Goldman Sachs (2007) recently cited *Jatropha curcas* as one of the best candidate for future Bio-fuel production.

2.2 Origin and Distribution of *Jatropha*

The origin of *Jatropha curcas* is uncertain but believed to have originated from tropical America; likely Mexico and Central America in the year 1600. From Central America it was spread by Portuguese traders as a valuable hedge plant via the Cape Verde Islands (an island country lying about 644 km off the coast of Senegal in West Africa) and former Portuguese

Guinea (Guinea Bissau) (Henning, 2008) to countries in Africa, Asia and India (Jepsen *et al*., 2006). It was tested for fuel production by the French in Mali and also by the Portuguese on Cape Verde in 1955 (Bird, 1957). In Tonga (independent island nation in the southern Pacific Ocean, located approximately 650 km southeast of Fiji), it often grows in rows around plantations, along roads, and other places where it is used as life fence posts (Yuncker, 1959).

In Nigeria, the plant is widely found in the country which is mostly used for its medicinal purpose and fencing of farms and houses. *Jatropha curcas* is widely cultivated as an ornamental plant which in its natural distribution area grows in arid and semi-arid conditions (Makkar *et al*., 1997; Openshaw, 2000). It is also adapted to a wide range of climatic and edaphic conditions, but its strength as a crop comes from its ability to grow on harsh, dry sites (gravelly, sandy, stony and saline soils as well as crevices of rocks). It grows best on well drained soils with good aeration though adapted to marginal soils with low nutrient content. The current distribution shows that introduction has been most successful in the drier regions of the tropics with annual rainfall of 300-1000 mm per annum. It is reported to be very drought tolerant and can withstand slight frost (Agro Forestry Tree Database, 2007). *Jatropha curcas* grows best mainly at lower altitudes (0-500 m above sea level), in areas with average temperature of 20-28º C. The leaves shed during the winter/dry months and form mulch around the base of the plant which serves as organic matter and thus enhances the activities of soil microbes around the root-zone of the plant which helps in improving the fertility and structure/texture of the soil. *Jatropha curcas*, occasionally, is grown in the warmer parts of Australia and it is naturalized in few places in Queensland and Northern

territory. In Florida it is found chiefly in South Orlando. It is also common in Hawaiian Islands.

2.3 Production of *Jatropha curcas*

The Jatropha plant, once planted in the field starts fruiting after two years and continues up to 30-50 years. Therefore, the freshly harvested seeds from the identified quality planting material having desirable characteristics like high seed yield, high oil content in seed, synchronized maturity and resistance to insect pests and diseases should always be used for raising of nurseries. The use of fresh seeds improve germination, as does seed treatments such as pre-soaking, drying and partial removal of the seed coat (Agro Forestry Tree Database, 2007). Dagar *et al* (2004) found that germination of *Jatropha curcas* seeds was highest at a soil pH of 8 (90% germination) and most rapid at a soil pH of 6–8 (six days to maximum germination). In moist soils, germination takes 10 days (Agro Forestry Tree Database, 2007).

Jatropha curcas is easily cultivated by direct seeding, transplanting of seedlings and from cuttings (Agro Forestry Tree Database, 2007). Seeds for sowing are collected after the capsules split open. Sowing by seed is mostly encouraged because plants propagated by seeds have a longer lifespan of 50 years or more in relation to those propagated by cuttings which have a shorter lifespan of 15 years or less (Nyamai and Omuodo, 2007). Seedlings are raised in the flat seed beds or in polyethylene bags of 0.5 kg capacity filled with mixture of soil and farm yard manure (FYM) in the ratio of 4:1. Although *Jatropha curcas* is said to grow in marginal soils, it is not a nitrogen fixing plant and hence require nitrogen rich soils for good production (Van Ejick, 2006). Two seeds are sown in each polyethylene bag at a

depth of 1.5-2.0 cm and watered regularly. When the seedlings are about 4 weeks old, the weaker ones can be removed and used for gap filling. The seeds can also be directly planted on the field; the land is ploughed once or twice depending upon the nature of soil. In case of heavy soils, deep ploughing is desirable, whereas in light soil shallow ploughing is sufficient. The plants can also be raised easily through cutting. Cuttings should be from one year old branches and should be 5 cm in diameter, 25 cm long and with 4 to 5 buds (Singh *et al*., 2007).

In Nigeria, February-March is the best time for sowing of nursery and July-August (rainy season) is the best time for transplanting seedlings in the field. Transplanting can be done when the healthy seedlings are four weeks old and should be planted in a hole at the required depth (30 cm × 30 cm) and filled with a mixture of soil, farm yard manure (2-3 kg.) and fertilizer (20 g urea, 120 g single super phosphate and 16 g muriate of potash). Spacing in plantations can range from 2m × 1.5m to 3m × 3m (Jones and Miller, 1992). The number of trees per hectare at planting will range from 1600-2200. Non-economically wider spacing was reported to give larger yield of fruit, 79 kg/ha and 318 g/shrub (Heller, 1996). *Jatropha curcas* begins to produce fruits at 2 years of age and reaches maximum seed production at about three years of age (Agro Forestry Tree Database, 2007). To give a bushy shape the plant should be pruned during spring (February-March in Nigeria) up to 5years including one pruning when the plants attain 1.5 m height. The terminal-growing twig is to be pruned to induce secondary branches. Likewise, the secondary and tertiary branches are to be pruned at the end of first year to induce a minimum of 25 branches at the end of second year. Once in ten years, the plant may be cut leaving one foot height from ground level for

rejuvenation. The growth is quick and the plant will start yielding in about a year period. This will be useful to induce new growth and yield stabilization thereafter (Agro Forestry Tree Database, 2007).

2.4 Economic Importance of *Jatropha curcas*

Special interest has been shown in the cultivation of the tropical physic nut for oil extraction (Grimm, 1996; Heller, 1996). It has been reported that the oil can be combusted as fuel without being refined; it burns with clear smoke free flame and it was successfully tested as fuel for simple diesel engine. As the world focus moves toward green and clean energy source, this wonder plant will be a very good candidate because it grows in degraded land hence; the rational production of biodiesel from *Jatropha curcas* would not compete with human food security (Ovando-Medina *et al*., 2009). If *Jatropha curcas* is cultivated as fence or intercropped, the problem of pest and diseases is not very significant and can easily be controlled, but when cultivated in large-scale monoculture, pests and diseases may become severe (FACT, 2007). There is concern that, for instance, *African cassava mosaic virus* may be transmitted to *Jatropha curcas*, although cases have only been reported in *Jatropha multifida*. Münch (1986) states that cassava super elongation disease *(Sphaceloma manihoticola/Elsinoe brasiliensis*) can be transmitted to *Jatropha curcas*. For these reasons it is advised not to plant cassava and Jatropha in the same field (Heller, 1996). Common bean (*Phaseolus vulgaris* L.) is susceptible to *Jatropha mosaic virus* (Hughes and Shoyinka, 2003) which is transmitted by whitefly (*Bemisia tabaci* Genn.) (Raj *et al*., 2008a). In Nigeria,physic nut is called “cin da zugu” (Hausa), “wulu-idi” (Igbo) and “lapalapa” (Yoruba).

The proximate composition of the physic nutseeds is Moisture, 6.20%; Protein, 18.00%; Fat, 38.00%; Carbohydrates, 17.00%; Fiber, 15.50% and Ash, 5.30%. Oil content is about 35 to 40% in the seeds and 50 to 60% in the kernel. The oil contains 21% saturated fatty acids and 79% unsaturated fatty acids. The composition of *Jatropha curcas* oil consists of mainly fatty acids such as palmitic 18.22%, stearic 5.14%, oleic 28.46% and linoleic 48.18% (Jumat and Rozaini, 2008).

Currently the oil from *Jatropha curcas* seeds is used for making biodiesel fuel. Likewise, physic nut plant is being promoted as an easily grown bio-fuel crop in hundreds of projects throughout the world. In Africa, cultivation of *Jatropha curcas* is being promoted and it is grown successfully in countries such as Mali, South Africa, Kenya and Nigeria. Among the benefits of *Jatropha curcas* cultivation that government is trumpeting are the reduction in air pollution and the country's dependence on "imported" crude oil, construction of roads, bridges, refineries as well as job creation.

On Dec. 30, 2008 Air New Zealand successfully completed a test flight from Auckland using a 50/50 mixture of Jatropha oil -derived bio-fuel and Jet A1 in one of the four Rolls-Royce RB211 engines of a 747 jumbo jet. Air New Zealand announced plans to use the new fuel for 10% of its needs by 2013 (www.enviro.aero). At the time of this test, *Jatropha curcas* was significantly cheaper than crude oil, costing an estimated $43 a barrel or about one-third of the June 4, 2008 closing price of $122.30 for a barrel of crude oil (www.oilprice.com). Cars and other machineries are at various stages of testing with physic nut bio-fuel in order to reduce the impact of climate change as a result of global warming.

The most exploited uses of oil pressed from the seed in rural-industrial development include lubrication, soap and candle making (Tigere *et al*., 2006), as well as adulteration of olive oil, and making Turkey red oil. Turkey red oil, also called sulphonated (or sulfated) castor oil, is the only oil that completely disperses in water. It is used in formulating lubricants, softeners, and dyeing assistants. In China, the oil is used to produce furniture varnish after boiling it with iron oxide (Ochse, 1980). The seed oil is also reported to possess insecticidal, mollusicidal, fungicidal and nematicidal properties (Emeasor *et al*., 2005; Abdel-Hamid, 2003; Lakshmanan *et al.*, 1990). Jatropha oil cake contains about 6 % nitrogen, 2.75 % phosphate and 0.94 % potassium and thus can be used as organic manure (Makkar *et al*., 2001) as it contains more nutrients than chicken and cattle manure.

In Nigeria, substitution of firewood by plant oil for household cooking in rural areas will not only alleviate the problems of deforestation but also improve the health of rural women who are subjected to the indoor smoke pollution from cooking with inefficient fuel and stoves in poorly ventilated space (Abdulkadir, 2010). The seed cake or seeds can be used as animal feed (Makkar *et al.,* 2001).The cakes remaining after the oil has been pressed out can also be used as domestic fuel, fertilizer and animal fodder. Other uses of *Jatropha curcas* include; the young leaves of *Jatropha curcas* may be steamed or stewed (Ochse, 1980) and can also be cooked with goat meat and this is said to advantageously counteract its smell. The seeds in the zone around Misantla, Veracruz are very appreciated as food once they have been boiled and roasted (cited by Henning, 1997). It is unclear if this is due to the existence of a non-toxic variety of *Jatropha curcas* in Mexico and Central America, or if the seeds become edible once processed by cooking. Pounded leaves are applied near horses' eyes to repel flies

in India (www.purdue.edu) and also used as tea against many diseases such as malaria in Nigeria (Abdulkadir, 2010). The nuts are sometimes roasted and eaten, although they are purgative and can be burned like candlenut when strung on grass (Watt and Breyer-Brandwijk, 1962). It has been used as a contraceptive in South Sudan (List and Horhammer, 1969). Seed husks can be used as domestic fuel generators.

The root ashes are used as a salt substitute (Morton, 1981). The bark of the plant produces a dark blue dye, which is used for colouring cloth, fishing nets and lines (Singh *et al*., 2007). It can also be used as a fish poison (Watt and Breyer-Brandwijk, 1962). The latex strongly inhibits the watermelon mosaic virus (Tewari and Shukla, 1982). According to Agaceta *et al*. (1981), the latex can be used as a remedy for alopecia, anasorca, burns, dropsy, eczema, inflammation, paralysis and yellow fever as well as for wound healing in Nigeria. It is also used to treat various skin diseases and rheumatism (Heller, 1996). The sap is used in staining linens as well as markings (Mitchell and Rook, 1979). The emulsified oil has been found to be an effective insecticide against weevil pests and houseflies and an oil extract has been found to control cotton bollworm and sorghum stem borers (Gubitz *et al*., 1999). Shanker and Dhyani (2006) and Achten *et al*. (2008) described the use of oil extracts as an insecticide, molluscicide, fungicide and nematicides.

In Nigeria, *Jatropha curcas* is planted in the form of hedges around gardens or fields to protect the crops against roaming animals like cattle or goats as well as used as a shade for coffee, tomato, pepper etc. nurseries, demarcate the boundaries of fields and homesteads as well as erosion and desertification control (Abdulkadir, 2010).

2.5 Insect Pests and Diseases of *Jatropha curcas*

Contrary to popular belief that toxicity and insecticidal properties of *Jatropha curcas* are a sufficient deterrent for insects that cause economic damage in plantations, several groups of insects have overcome this barrier. Particularly noteworthy is the insect order Heteroptera which can extract nutrients from physic nut (Grimm and Maes, 1997). The key insect pests of economic importance identified are *Scutellera nobilis, Pempelia morosalis, Bemisia tabaci, Stomphastis thraustica, Oxycetonia versicolor, Archaea janata* (Shanker, 2006).

In Australia where *Jatropha curcas* is considered as a weed, the insect that caused maximum damage proved to be the seed-feeding Scutelleridae, *Agonosoma trilineatum* (Smith and Heard, 2003).The crop is projected to have less pest damage and hence, minimum requirement for plant protection (Shanker, 2006). However, in reality, even in India where *Jatropha curcas* is grown in continuous stretches as a monocrop, it is devastated by two insect pests that are emerging as a major problem in *Jatropha curcas* cultivation- the scutellarid bug, *Scutellera nobilis* Fabr. which causes flower fall, fruit abortion, and malformation of seeds (Shanker, 2006). The inflorescence and capsule-borer, *Pempelia morosalis* also causes economic damage by webbing and feeding on inflorescences and in later stages boring into the capsules. Other pests that have caused noticeable damage are the blister miner,(*Stomphastis thraustica* Meyerick.), the semi-looper, *Archaea janata*, brown soft scale (*Coccus hesperidum* Linnaeus.), Mealybug (*Paracoccus marginatus* Williams.), Red spider mite (*Tetranychus* sp.), Termites (*Odontotermes formosanus* Shiraki.) and to a small extent, the flower beetle (*Oxycetonia versicolor)* (Ranga Rao *et al.* 2010).

Various diseases caused by plant pathogens have been reported on *Jatropha curcas*. These diseases arespots on seedlings, dieback, Alternaria leaf spots caused by *Alternaria ricini,* Rust caused by *Melampsora ricini,* Fusarium wilt caused by *Fusarium oxysporum* (FACT, 2007) and damping off caused by *Phytopthora* spp. (Jongschaap *et al.*, 2007),

2.6 Viruses of *Jatropha curcas*

2.6.1 *Cucumber mosaic virus* (CMV) genus *Cucumovirus*

The *Cucumber mosaic virus* is a ubiquitous virus which has been reported to be found during several virus surveys (Migliori and Cadihlac, 1976; Hughes *et al.*, 1997) in West Africa. *Cucumber mosaic virus* is efficiently transmitted in a non-persistent manner by aphids to a wide range of indicator plants (Kenyon *et al.*, 2003) that include *Chenopodium amaranticolor, C. quinoa, Cucumis sativus and Vigna unguiculata.* Symptoms exhibited by *Cucumber mosaic virus* include severe chlorosis and mosaic, leaf distortion and stunting. Apart from cucumber and other cucurbits, it is also reported to infect *Jatropha curcas* spinach, lettuce and celery and many flowers, especially lilies, delphiniums, primulas and daphnes as its host plant species (Hughes *et al.*, 2004)

2.6.2 *Jatropha Mosaic Virus*.

Jatropha mosaic virus (JMV) belongs to the Family *Geminiviridae* and genus Begomovirus which infects a wide range of dicotyledonous plants. The natural occurrence of a mosaic disease was noticed on *Jatropha curcas* growing in India (Raj *et al.*, 2008b). The disease incidence was significant and the symptoms of the disease were severe with leaf mosaic and reduced size, and stunting of whole plants. Transmission of the disease could be by whitefly

(*Bemisia tabaci*) in a persistent manner but it could not be transmitted through mechanical inoculations of sap from an infected plant unto jatropha seedlings (Raj *et al.,* 2008b). Kashina *et al.* (2013) reported the complete nucleotide sequence of DNA-A of a begomovirus naturally infecting *Jatropha curcas* L. in Nigeria. Symptoms observed were severe mosaic, mottling and blistering of leaves.

Other viruses reported to be infecting *Jatropha curcas* are *Cassava mosaic virus*, *African cassava mosaic virus* and *East African cassava mosaic virus*.

2.7 Criteria for Diagnosis of viruses

Many criteria such as symptomatology, host range, and serology are employed in routine virus identification. Some of these criteria employed for the identification of *Jatropha mosaic virus* are discussed here.

2.7.1 Symptomatology of *Jatropha mosaic virus*

Bird (1957) described the Jatropha mosaic disease with typical mosaic symptoms reported for the first time on *Jatropha gossypiifolia* L. in Puerto Rico (USA). The infected plants produced mosaic symptoms, vein thickening, laminar restrictions and leaf enations (Plate 1) on whitefly transmitted *J. gossypiifolia* L. affected by Jatropha mosaic begomovirus (Bird and Maramorosch, 1978). Similar types of symptoms on *J. multifida* L were observed in Puerto Rico (Kim *et al*., 1986). The JMV infected plants produced similar type of symptoms within two weeks after inoculation with whitefly (*Bemisia tabaci*) (Kim *et al.,* 1986). Brown and Bird (1992) described in detail the symptoms of mosaic disease on common weed *J. gossypiifolia* L. infected with whitefly (*B. tabaci*) transmitted *Jatropha mosaic virus*

in Puerto Rico (USA). The infected plants exhibited foliar malformations, leaf curling, leaf enations and brilliant yellow mosaic symptoms. The realization of the full potential of *Jatropha curcas*is however, threatened by the widespread prevalence of a disease that causes severe mosaic, mottling, blistering symptoms on *Jatropha curcas* leaves, leading to stunting, low productivity and eventual death of affected plants (Kashina *et al.,* 2013). Raj *et al.* (2008b) reported that mosaic, reduced leaf size caused stunting symptoms on infected *Jatropha curcas* as compared with healthy plant.

Ramkat *et al*. (2011) reported *Jatropha curcas* plants growing in the field showed symptoms ranging from reduced leaf size, malformation and severe dwarfing of 1-3 years old plants. Symptoms on *Jatropha curcas* plants growing in the glass house were registered after 3 weeks as severe leaf size, mild to severe chlorotic specks and chlorosis in some plants. Drying (like burning) and rolling of leaves from tips was observed on 3 months old cuttings. In cassava, the symptoms observed in plants, grown and maintained in the glasshouse were similar. Severe reduction, distortion of leaves and stunted growth of some plants occurred.



Plate I: Different type of symptoms on infected *J. curcas* leaves (a) Initiation of mosaic (b) Mild mosaic (c) Mottle-mosaic (d) Severe mosaic and distortion of leaf (e) Crinkling and vein swelling (f) Yellow patches.

Source: www.google.com

2.7.2 Host range of *Jatropha mosaic virus*

Host range is a list of the host of a parasite. The concept is essential to understanding the role of additional host in the perpetuation or establishment of plant pathogens (Alegbejo, 1978). The host range of a given virus is dependent on many factors including environment, experimental conditions, host variety, virus strain and the concentration of the virus of the host at the time of harvest for assay. In some weeds, viruses produce no symptoms, but these weeds can still act as a source of infection. *Jatropha mosaic virus* was first reported in a wild species of *Jatropha gossypiifolia* from Pueto Rico by Bird (1957). Symptoms of this disease persist in the following hosts: *J. podagrica, J. multifida, Croton lobatus, Jacquimontia tamnifolia, Phaseolus vulgaris, Manihot esculenta, Cucumis sativus*-yellow mosaics (Grewal, 1988; Gautam, 1990).

2.7.3 Detection of *Jatropha mosaic virus*

Much of the potential usefulness of serology may depend upon adequate purification method which would enable the preparation of highly specific antisera and allow quantifying of antigen concentration for serological test (Alegbejo, 1978). Serological reactions are highly specific and sensitive. An appropriate serological test in conjunction with other studies helps in identifying virus and virus strains. They are used to distinguish related viruses which appear identical by all other available pests.

2.7.3.1 Polymerase chain reaction (PCR)

The PCR technique has been used to detect begomoviruses and other viruses in both plants and vectors. It is a rapid and simple means of producing relatively large numbers of copies of DNA molecules from minute quantities of source DNA material, even when the source

DNA is of relatively poor quality (Kashina, 2003). Since Geminiviruses replicate by a dsDNA intermediate, PCR techniques are adequate for diagnosis in this group (Rojas *et al.*, 1993; Mehta *et al.*, 1994a). This method is more sensitive than hybridization techniques and does not require the use of radioactivity (Pico *et al.*, 1996).

Rangaswamy *et al*. (2005) reported that a band of approximately 500 bp was consistently amplified from total DNA extracted from infected *Jatropha curcas* samples. Polymerase chain reaction has been employed to detect and establish association of begomoviruses through amplification of approximately 500 bp fragment of DNA-A of begomoviruses using Deng primers (Deng *et al*., 1994) viz Primer A (forward) and Primer B (reverse) corresponding to the nucleotide sequence 146-165 and 650-672 bp respectively, were used in polymerase chain reaction to amplify DNA fragment of DNA-A to yield a product size ranging from 500 to 527 bp. This clearly indicates the association of a whitefly-transmitted geminivirus with the mosaic disease on *Jatropha curcas*. The association of JMV was confirmed through amplification of approximately 575 bp fragment of begomoviral coat protein gene using degenerate primers V324 and C889 (core Coat Protein) capable of universally amplifying begomovirus (Brown *et al.*, 2001). There are already reports on occurrence of *Jatropha mosaic virus* (JMV-Puerto Rico) on *Jatropha gossypifollia and Jatropha multifida* from Puerto Rico (USA) (Bird, 1957; Brown *et al.*, 1999). Wyatt and Brown (1996) detected JMV isolates from Puerto Rico using another set of degenerate primers (AV 494 and AC1048) directed to amplify the core region of coat protein DNA fragment of approximately 550bp.

When symptomatic *Jatropha curcas* samples from Kenya were tested with primer pair JC3F and JC4R, 69 % were positive as reported by Ramkat *et al.* (2011). The primers were able to further detect virus in 67 % of asymptomatic samples. For symptomatic *Jatropha curcas* samples collected from Ethiopia, 61 % tested positive with the primers JC6F and JC2R. With the same primers, 75 % of symptomatic samples from Kenya tested positive while 20 % of asymptomatic samples were detected positive.

2.8 Management of *Jatropha mosaic virus*

It has been recognized that management of the whitefly-geminivirus complex demands a considerable amount of collaboration and coordination of research worldwide in developing sustainable management systems to control the whitefly insect vector and the geminivirus pathogens. Management strategies of plant diseases caused by whitefly-transmitted (WFT) *Geminivirus* pathogens must be based on an understanding of the mechanism underlying successful interaction between the components of these virus-vector host complexes (Brown, 2000). Studies must also be involved in *Geminivirus* diversity, worldwide, with respect to viral genotype and phenotype, as these characteristics impact the molecular epidemiology of WFT *Geminivirus* and genetic engineering to complement traditional breeding in development of resistance to *Geminivirus*. Some of the management practices that can be applied are: planting of virus free seeds and transplants; use of resistant and tolerant varieties; management of vector population below damage level which can be achieved by application of various physical, cultural and chemical control methods. It must be recognized that the use of pesticides can lead swiftly to population resurgence, as they destroy the natural enemies and stimulate the fertility of *B. tabaci*. Alternating the type of

insecticide applied can serve to prevent or delay the development of pest resistance to a specific chemical. Finally, pest management practices in limiting the most destructive whitefly geminivirus complex on several crops can best be developed through the farmer participatory methodology for improved crop production.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Survey and Sample Collection

Surveys were carried out from August to September 2015 in selected *Jatropha curcas* fields at several locations covering three Local Government Areas (LGAs) each in Kaduna ;Sabon-Gari, Igabi and Jema'a (Figure 1), Kano; Minjibir, Tudun-Wada and Wudil (Figure 2), and Katsina; Batagarawa, Dan-Musa and Charanchi (Figure 3) States, Nigeria to determine *Jatropha mosaic virus* disease incidences. The disease incidence at each location was assessed by counting the number of infected plants (showing typical *Jatropha mosaic virus* disease symptoms) of the total plants observed. The incidence of JMV was expressed as a percentage of diseased plants as stated by Chaube and Phundir (2005).

$$\text{Disease incidence (\%)} = \frac{\text{number of infected plants}}{\text{total number of plants assessed}} \times 100$$

Leaf samples (both symptomatic and non-symptomatic leaves) one each from the top, middle and base of each plant were sampled in a quadrant of 12×12 m^2 (3 quadrants marked at 2 corners and centre of the field), labelled, put in a cooler containing ice pack and transported back to the Plant Virology Laboratory, Department of Crop Protection, Ahmadu Bello University, Zaria, to be used for molecular tests. Twenty symptomatic and non-symptomatic leaf samples in the ratio 15:5 respectively were collected within each quadrant from the top, middle and base of each plant in a field. Samples from each quadrant were bulked together and a representative sample of twenty (in the ratio 15:5) taken. Sixty

samples were collected per LGA, one hundred and eighty samples per State, with a total of five hundred and forty samples across the three States.

3.2 Determination of Alternative Hosts of the Virus

During the survey, leaf samples from symptomatic and asymptomatic weeds 5 m in and around the vicinities of the sampled farms were collected randomly, to ascertain their role as alternative/reservoir hosts of the virus. The number of weeds showing symptoms and the total number of weeds collected from each field were recorded. Identification of the weeds/plants was carried out at the Herbarium Unit of the Department of Biological Sciences, Ahmadu Bello University, Zaria.

3.3 Detection of *Jatropha Mosaic Virus*

3.3.1 DNA Extraction

Genomic DNA was extracted from symptomatic and asymptomatic *Jatropha* and weed leaves using the Cethyl Trimethyl Ammonium Bromide (CTAB) protocol described by Doyle and Doyle (1987). All required buffers were prepared and stored in the refrigerator (see appendix 1). Prior to starting extraction, 2 g polyvinylpyrrolidone (PVP) and 250 µl β-mercaptoethanol were added to 50 ml CTAB buffer. Twenty milligrams of leaf tissue was weighed and frozen in liquid nitrogen in laboratory mortar and ground with pestle, keeping tissue frozen all the time by adding liquid nitrogen while grinding. Mortars and pestles were sterilized before, during and after grinding by soaking in 3.8 % v/v sodium hypochlorite for 30 mins before rinsing in water and autoclaving for 4 hrs. The ground tissue samples were transferred into microcentrifuge tubes using sterilized spatula.

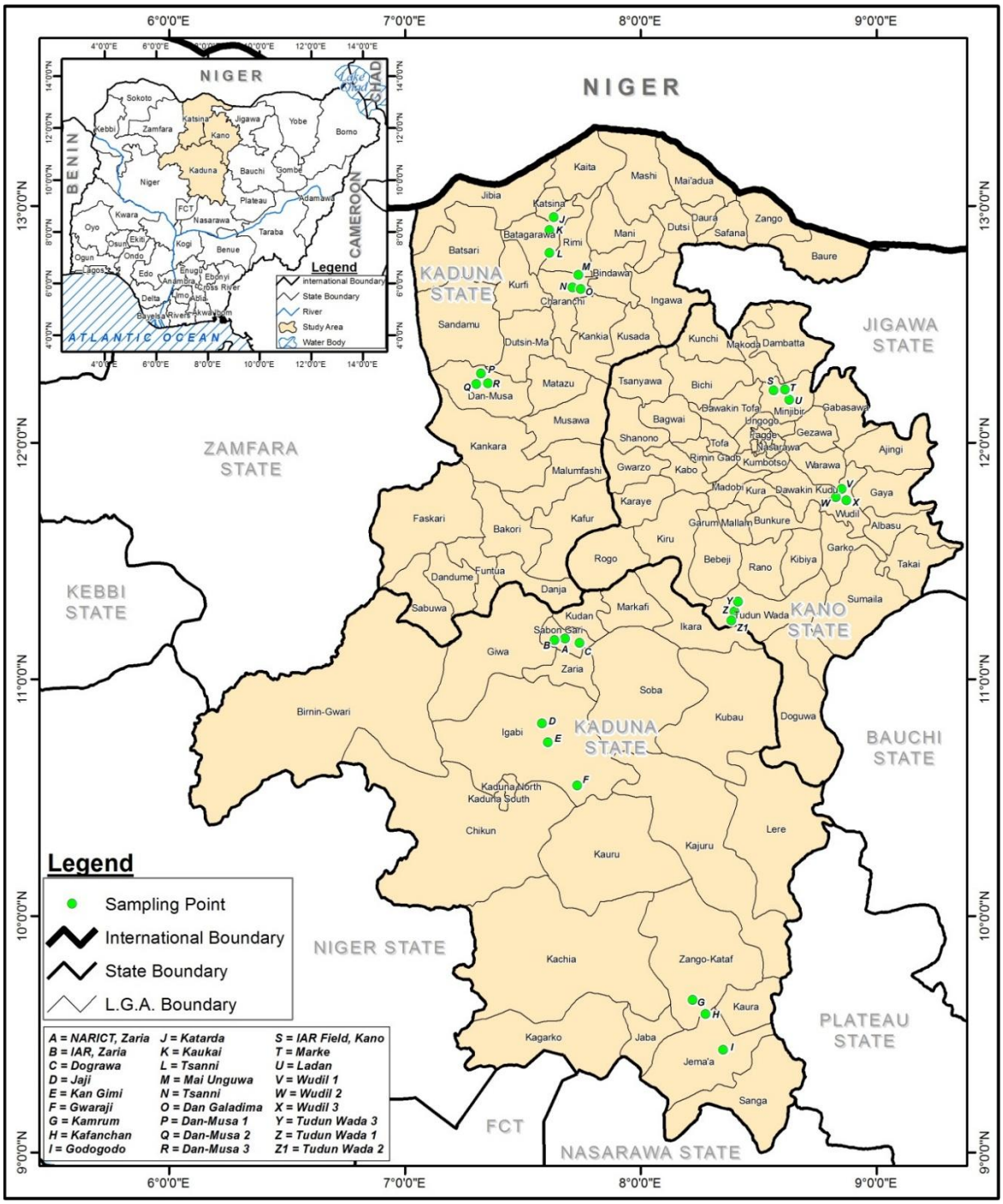


Figure 1: Map of Katsina, Kano and Kaduna States showing *Jatropha mosaic virus* disease sampling points.

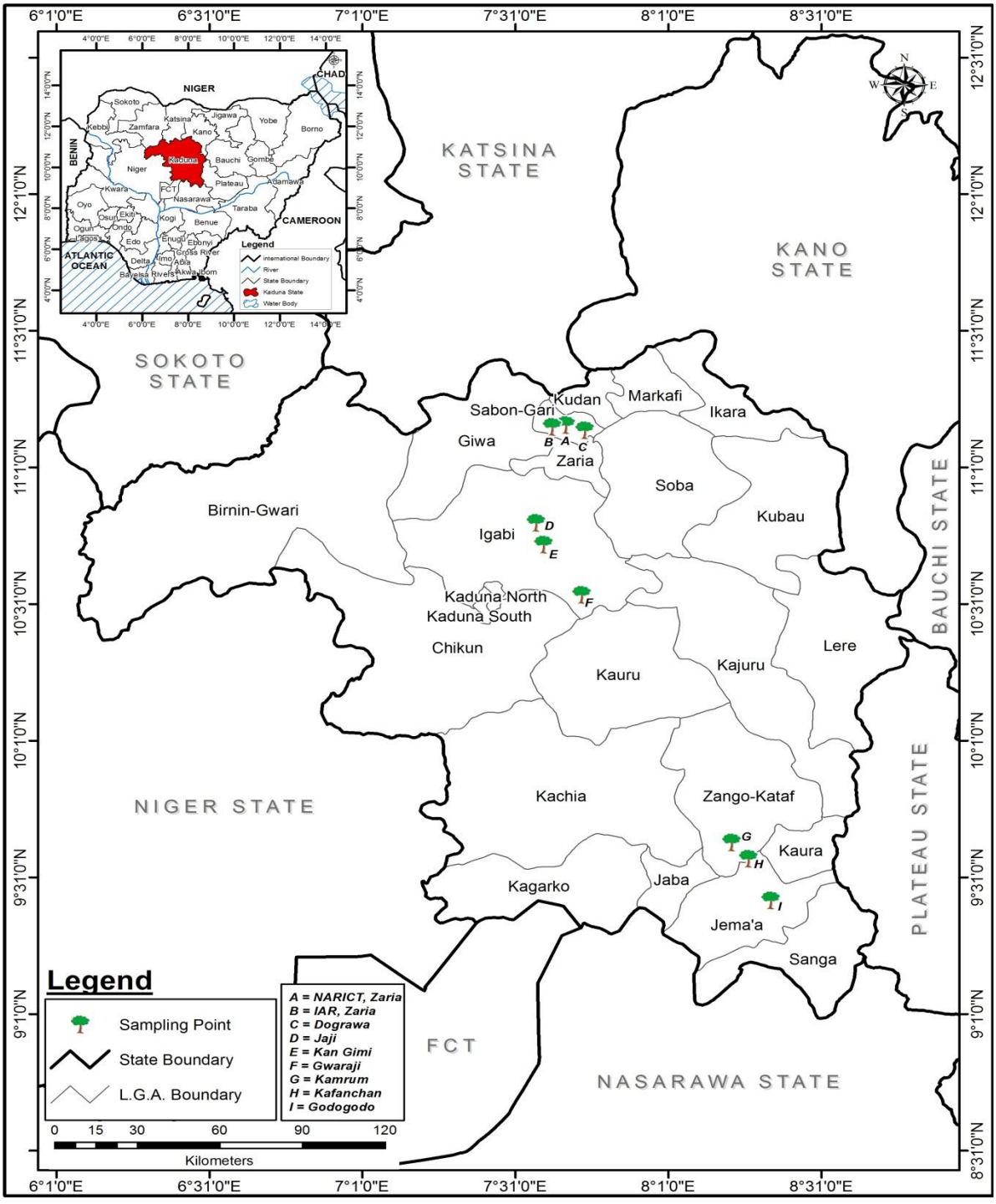


Figure 2: Map of Kaduna State showing *Jatropha mosaic virus* disease sampling points

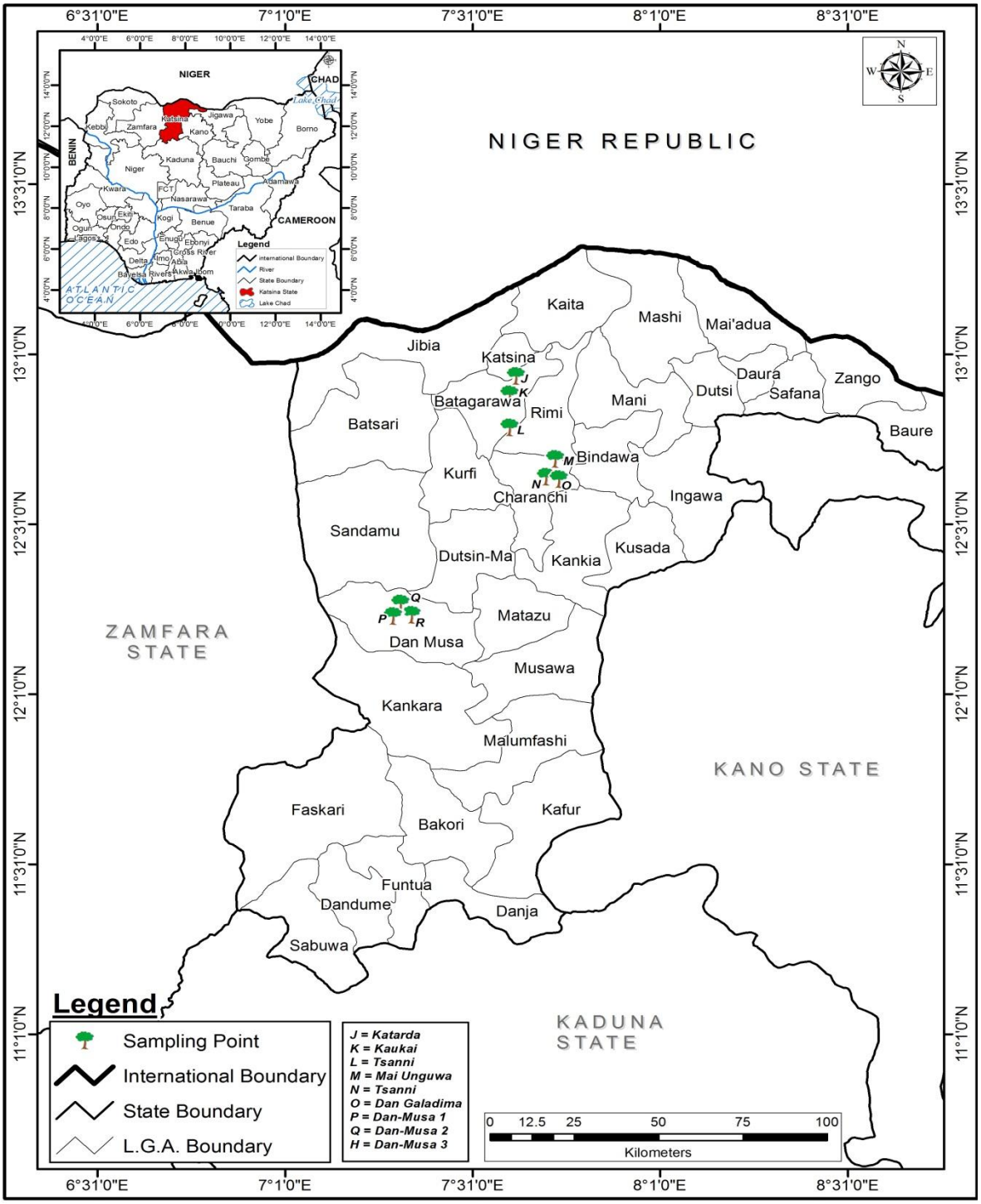


Figure 3: Map of Katsina State showing *Jatropha mosaic virus* disease sampling points

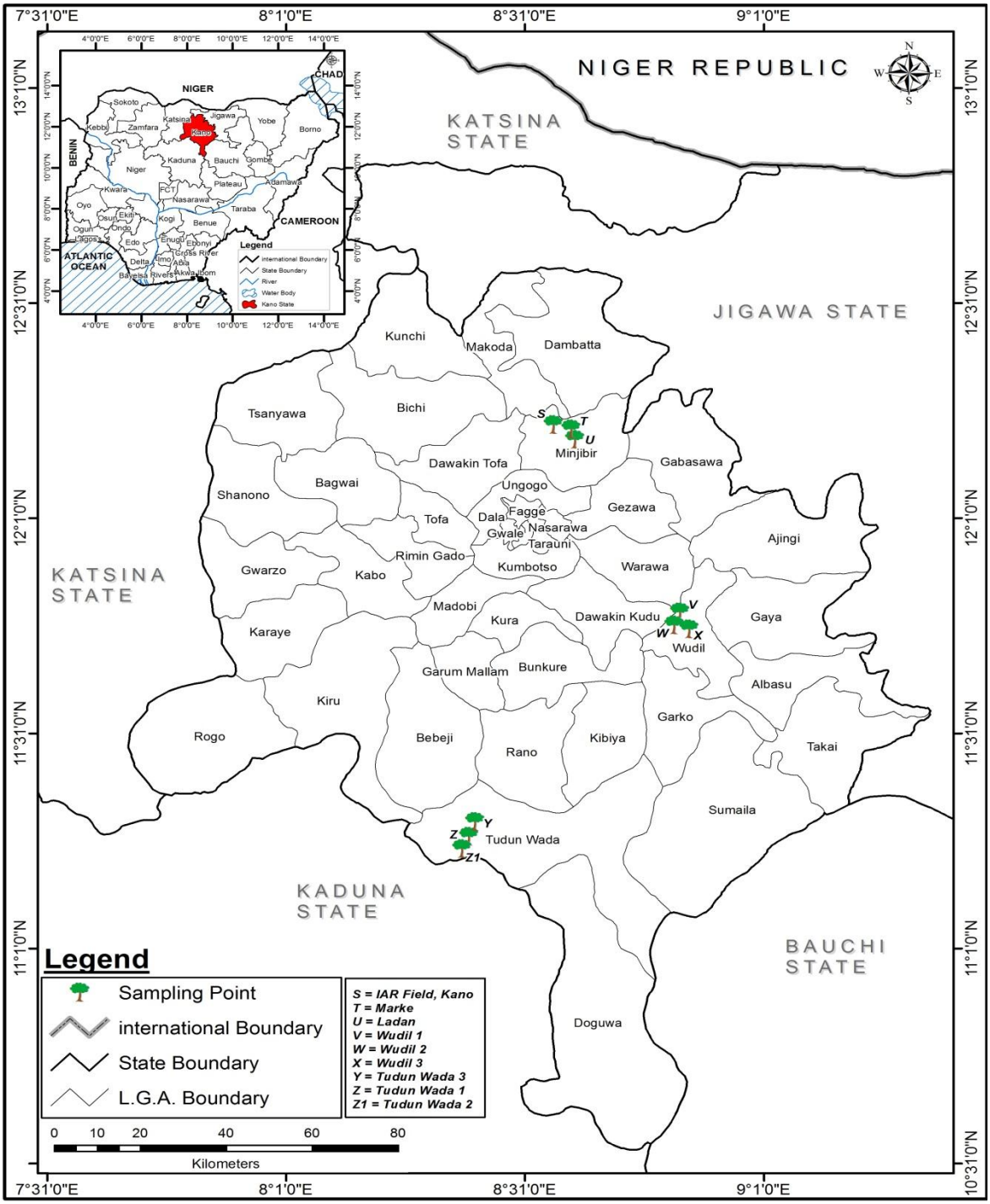


Figure 4: Map of Kano State showing *Jatropha mosaic virus* disease sampling points

Five hundred micro litres of CTAB buffer was added into the tubes and mixed by vortexing making sure the leaf tissue was in solution and not in a clump at the bottom of the tube. The samples were then incubated in a laboratory water bath for one hour at 55°C, mixing once after 30 mins. Afterwards, 500 µl of chloroform was added, mixed by gentle shaking and centrifuged for 7 mins at 16,000 rpm in a high speed centrifuge model TGL-14G. The aqueous phase (45–50 µl) was transferred into a new labelled microcentrifuge tube using a micro-pipette, 38 µl of cold 7.5 M ammonium acetate and 260 µl of cold isopropanol were added and mixed by inverting tubes. The mixture was then allowed to incubate on ice for 30 mins. The mixture was centrifuged for 3 mins at 16,000 rpm to pellet the DNA. Then 700 µl pure ethanol (95 %) was added and centrifuged for 1 min at 16,000 rpm. Thereafter, 700 µl of 70 % ethanol was added and incubated for 1 min at 16,000 rpm. The supernatant was discarded carefully not to dislodge pellets. The microcentrifuge tubes containing the pellets were then inverted on a clean serviette and allowed to dry for 10 mins upside down. Then they were allowed to dry upright but covered with serviette for 30 mins. Finally, pellets were hydrated with 50 µl TE buffer and allowed to re-suspend overnight at room temperature. The DNA was then stored in the freezer at -18°C for subsequent use. To test for the presence of genomic DNA, a test run using gel electrophoresis in a 1% gel for 80 mins at 120 V was performed on selected samples. The presence of visible bands on the gel indicated the presence of genomic DNA.

3.3.2 Polymerase Chain Reaction procedure

Polymerase chain reaction (PCR) was used for DNA amplification. Sample DNA was subjected to PCR using the specific forward and reverse primer sequence for each viral

component (Table 2). The PCR cocktail was as follows: 12.5µl 2× DreamTaq Master Mix, virus forward primer 1 µl (10 pM/ µl); virus reverse primer 1 µl (10 pM/ µl); DNA template 2.0 µl; 8.5 µl sterile distilled water; a drop of sterile mineral oil was layered on reaction mixture to prevent evaporation during the PCR process.

The PCR for each viral component was run for 30 cycles. DNA was initially denatured in one cycle at 94℃ for 5 min (DNA-A, B, β and 1) and amplified for 30 cycles (Peltier 200 Thermocycler); at 94°C, 1 min for melting; at 52°C (DNA-A and 1), 50°C (DNA-B), 55°C (DNA-β), 1 min for annealing and 72°C, 1 min for DNA extension. This was followed by a final extension 72°C for 5 min (Table 1).

The PCR products were analyzed by electrophoresis in 1.0 % agarose gels in 1× Tris-borate-EDTA buffer (Appendix I).

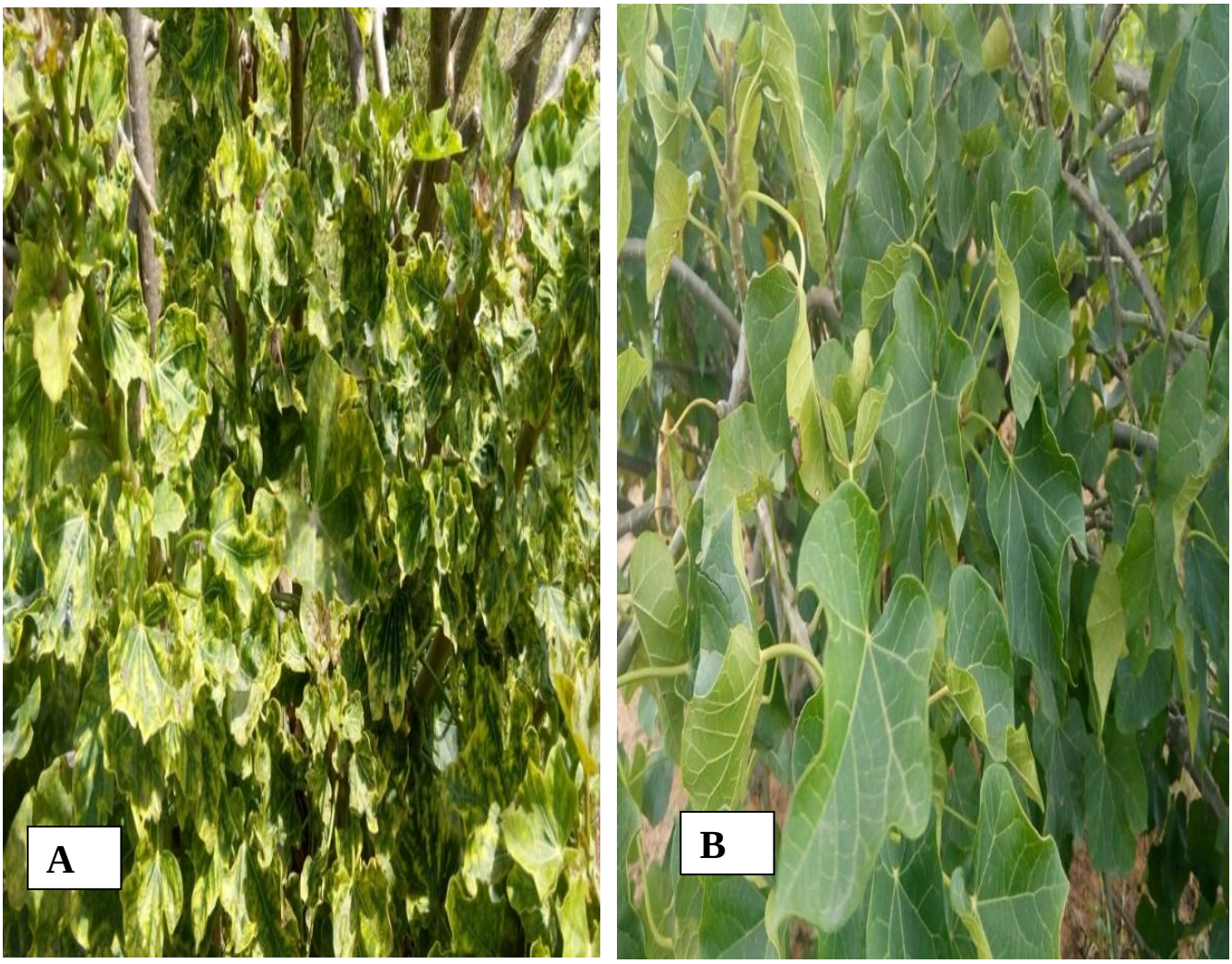


Plate II: Mosaic, leaf blistering and mottling on infected Jatropha plant (A) as compared with a healthy plant (B)

Source: Chindo (2015)

Table 1: PCR cyclic conditions for DNA-A, DNA-B, DNA-β and DNA-1

	30DNA-A cycles	**30DNA-B cycles**	**30 DNA-β cycles**	**30 DNA-1 cycles**
Initial denaturation	94ºC for 5min	94ºC for 5min	94ºC for 5min	94ºC for 5min
Denaturation	94ºC for 1min	94ºC for 1min	94ºC for 1min	94ºC for 1min
Annealing	52ºC for 1min	50ºC for 1min	55ºC for 1min	52ºC for 1min
Extension	72ºC for 1min	72ºC for 3min	72ºC for 1min	72ºC for 1min
Final extension	72ºC for 5min	72ºC for 5min	72ºC for 5min	72ºC for 5min

The PCR products were kept at 4°C in a refrigerator until when Agarose gel electrophoresis was carried out (Wyatt and Brown, 1996).

Table 2: Primers used for PCR analysis and sequencing

Primer	**Virus segment**	**Sequence (5'-3')**	**Orientation**	**Band sizes (bp)**
PAL1v1978	DNA A	GCATCTGCAGGCCCACATYGTCTTYCCNGT	Forward	500-600
PAR1c496	DNA A	AATACTGCAGGGCTTYCTRTACATRGG	Reverse	500-600
PBL1V2040	DNA B	GCCTCTGCAGCARTGRTCKATCTTCATACA	Forward	500-600
PCRc1	DNA B	CTAGCTGCAGCATATTTACRARWATGCCA	Reverse	500-600
Beta01	DNA β	GGTACCACTACGCTACGCAGCAGCC	Forward	300-500
Beta02	DNA β	GGTACCTACCCTCCCAGGGGTACAC	Reverse	300-500
NLDNA1-For	DNA 1	TGGTTYTATWCACGTGGHGG	Forward	300-500
NLDNA1-Rev	DNA 1	ARAWGATAGTKCKRTCATCTG	Reverse	300-500

Source: (Rojas*et al*., 1993). Primers were outsourced from Inqaba Biotech, South-Africa

3.3.3 Agarose gel electrophoresis

Agarose gel was prepared (1.0%) in 1×Tris-borate-EDTA (TBE) buffer (by dissolving 1g agarose in 100ml buffer) and boiling in a microwave oven with intermittent shake until all the agarose was completely dissolved. The edges of the gel tray were sealed with a tape and comb placed at one end of the tray surface. The agarose solution was allowed to cool to about 50ºC before pouring it into the gel tray to a thickness of 4-5mm, and then allowed to solidify. The tape was removed and the gel tray was then placed in the electrophoresis unit. TBE buffer was used to fill the unit so that there was 2-3mm of buffer over the gel surface. The comb was carefully removed and 6µl of loading buffer was mixed with 30µl of PCR product and 2µl loaded slowly into the wells. DNA molecular marker was also loaded. The order of sample loading in the gel was recorded. The unit was connected to a power jack and power supply turned on until the bromophenol blue dye reached the bottom of the gel (approximately 60min at 120V, for DNA to migrate 7cm from the wells in a 1.0% gel). The gel was removed from the tray and stained in ethidium bromide solution (0.5µg/ml) for 15min with gentle agitation. The gel was de-stained by briefly soaking the gel in distilled waterand observed under Ultra-Violet Transilluminator using UV full safety masks that efficiently blocked UV light. Photographs of the gel was taken (Kumar, 2009). The band sizes were checked against the 1 kb molecular marker for presence of geminiviral DNA-A and B components, as well as for sub viral components (DNA-β and DNA-1).

CHAPTER FOUR

4.0 RESULTS

4.1 Incidence of Jatropha Mosaic Virus

Jatropha mosaic virus disease was present in all farm locations and the incidences varied. The highest disease incidence in Kaduna State was recorded at NARICT Jatropha field with the highest incidence (83.61 %) in Sabon-Gari LGA (Figure 6), with the lowest at Gwaraji (42.86 %) in Igabi LGA (Figure 5). In Katsina State (95 %) was recorded at Walin-Hausa Foundation Farm (Tsanni) in Batagarawa LGA (Figure 13), and the lowest (43.75 %) in Katarda of Batagarawa LGA (Figure 13). The highest disease incidence (88 %) recorded in Kano State was at Tudun-Wada LGA (Figure 10), with the lowest (2 %) recorded in the Jatropha farm, IAR substation at Minjibir LGA (Figure 9). From the results, Katsina State recorded the highest disease incidence (71.97 %) overall (Figure 17).

4.2 Polymerase Chain Reaction

Of all the five hundred and forty samples collected, PCR was run successfully for 180 samples from all fields surveyed. The presence of a single medium-high molecular band on the agarose gel, indicates that DNA is amplified.

The specific primers were used to specifically amplify fragments of the geminiviral (DNA-A and DNA-B) and subviral (DNA-β and DNA-1) components of begomoviruses. An expected 0.3 - 0.5 kb fragment of DNA-β was amplified from the Jatropha samples of NARICT Jatropha field (N2, N3, N6, N8, N10, N12, N14, N16, N18, N20) and IAR Jatropha field (I5, I8, I10, I16, I20) in Kaduna State; however, amplicons using DNA-A, B and 1 specific primers were not detected (Plates 6-11), suggesting the presence of

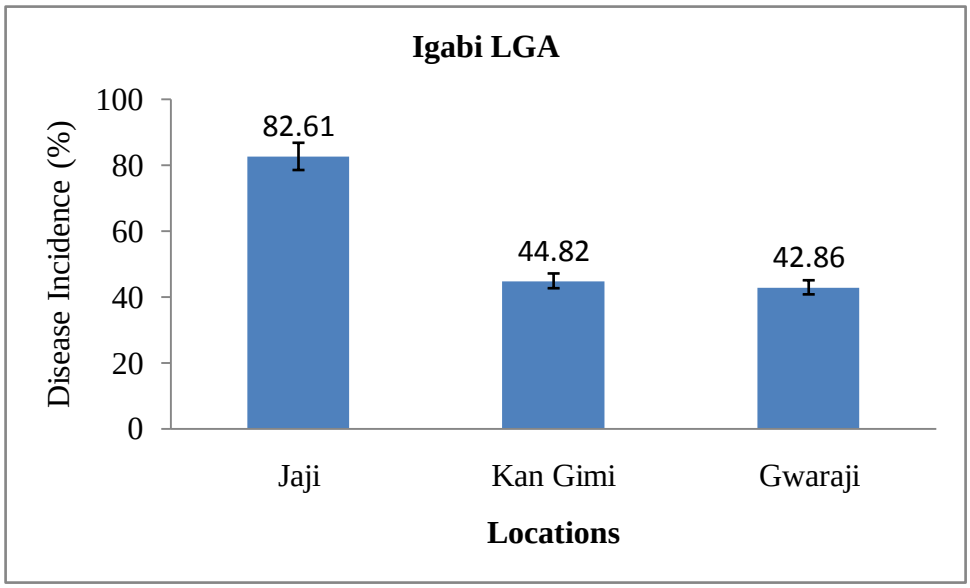


Figure 5: Incidence of *Jatropha mosaic virus* in Igabi Local Government Area of Kaduna State

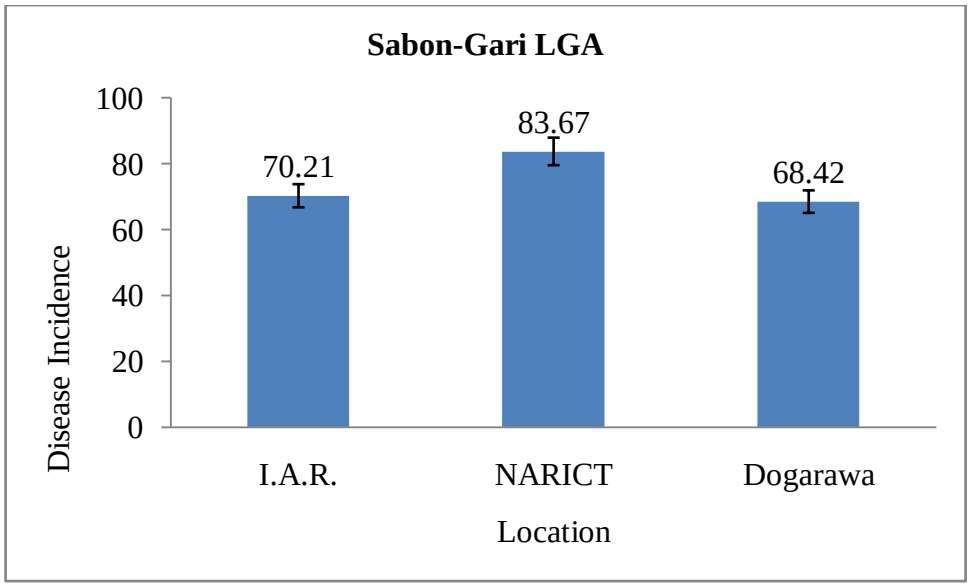


Figure 6: Incidence of *Jatropha mosaic virus* in Sabon-Gari Local Government Area of Kaduna State

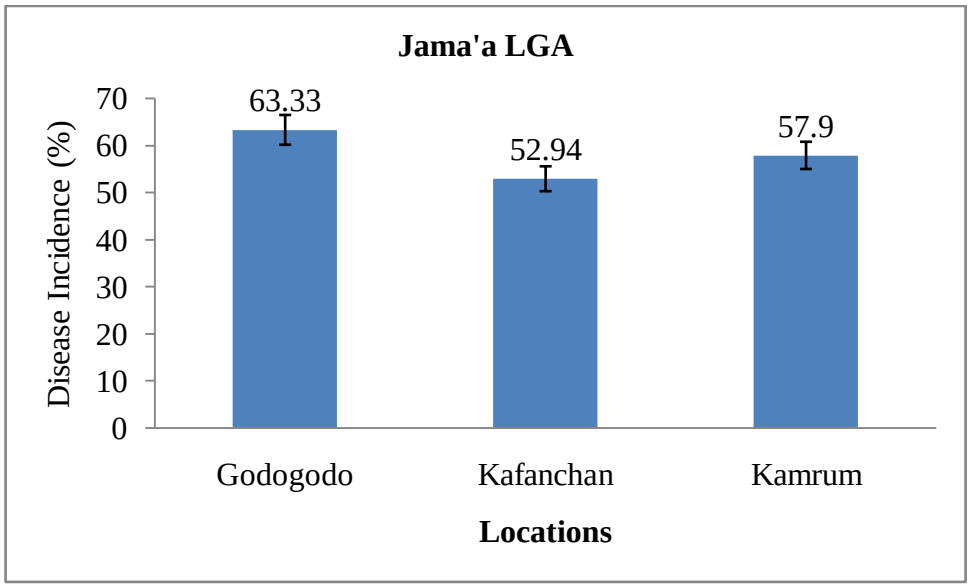


Figure 7: Incidence of *Jatropha mosaic virus* in Jama'a Local Government Area of Kaduna State

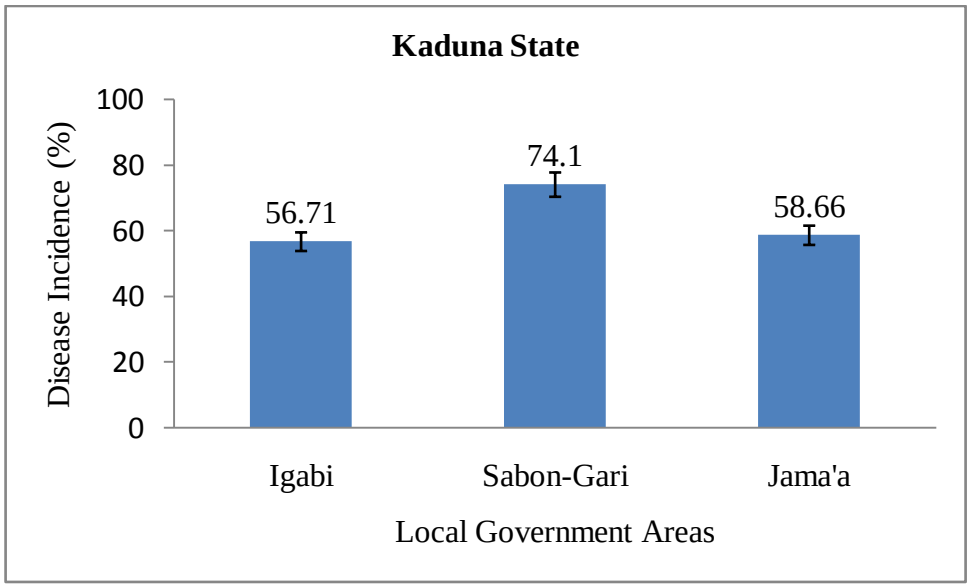


Figure 8: Incidence of Jatropha Mosaic Virus in Kaduna State

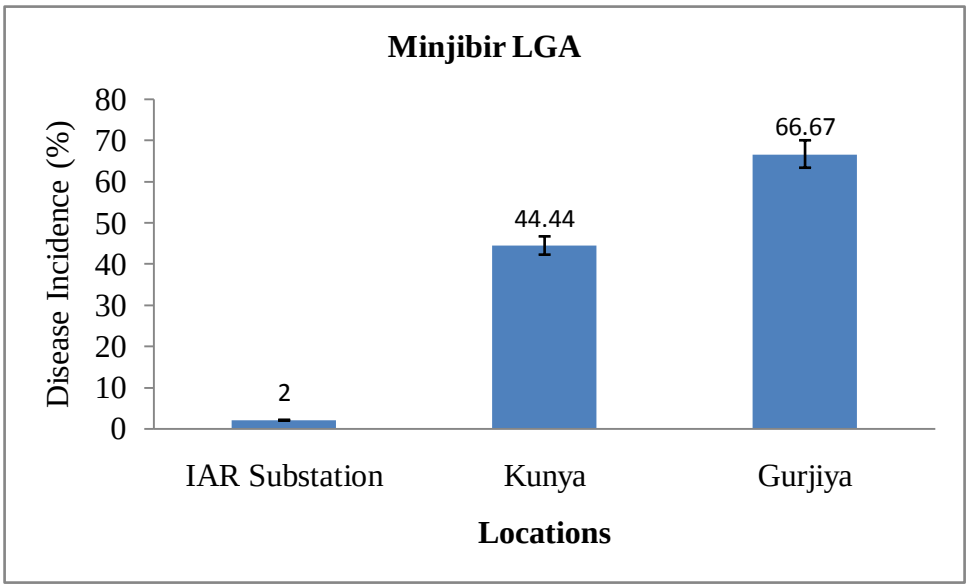


Figure 9: Incidence of *Jatropha mosaic virus* in Minjibir Local Government Area of Kano State

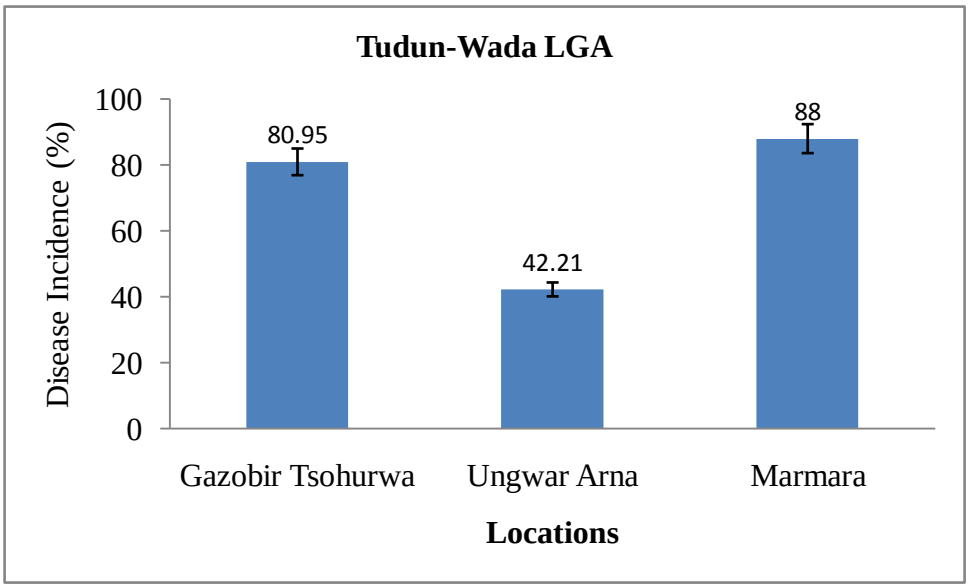


Figure 10: Incidence of *Jatropha mosaic virus* in Tudun-Wada Local Government Area of Kano State

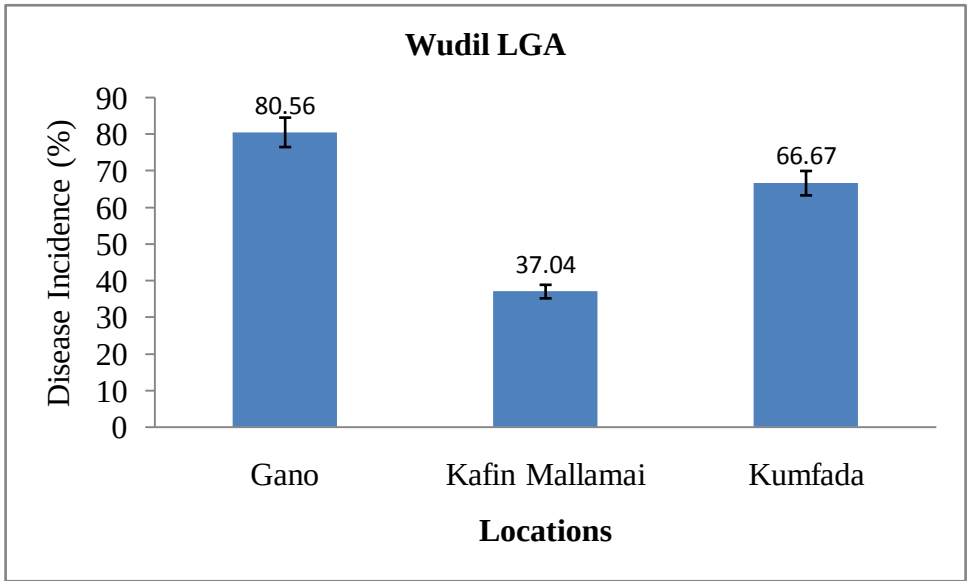


Figure 11: Incidence of *Jatropha mosaic virus* in Wudil Local Government Area of Kano State

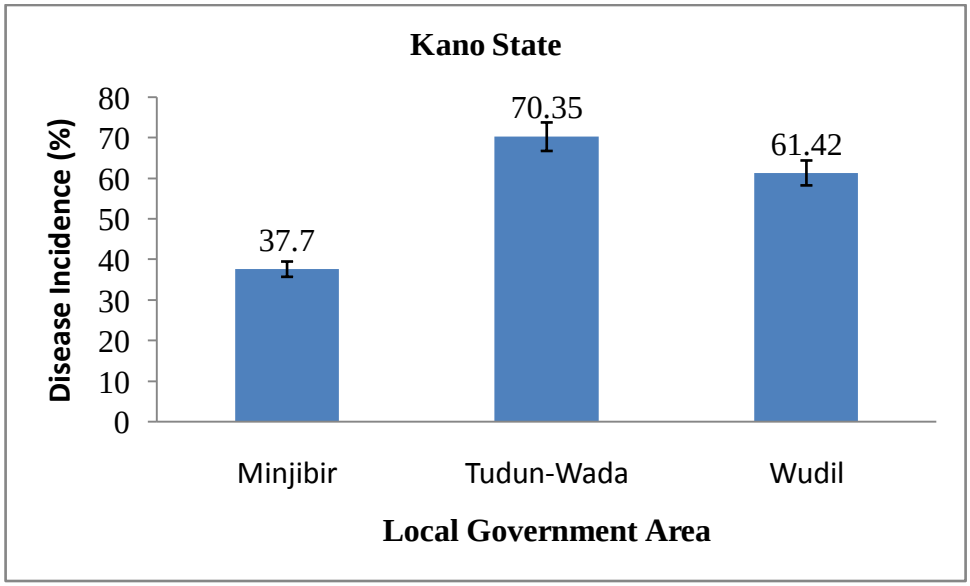


Figure 12: Incidence of *Jatropha mosaic virus* in Kano State

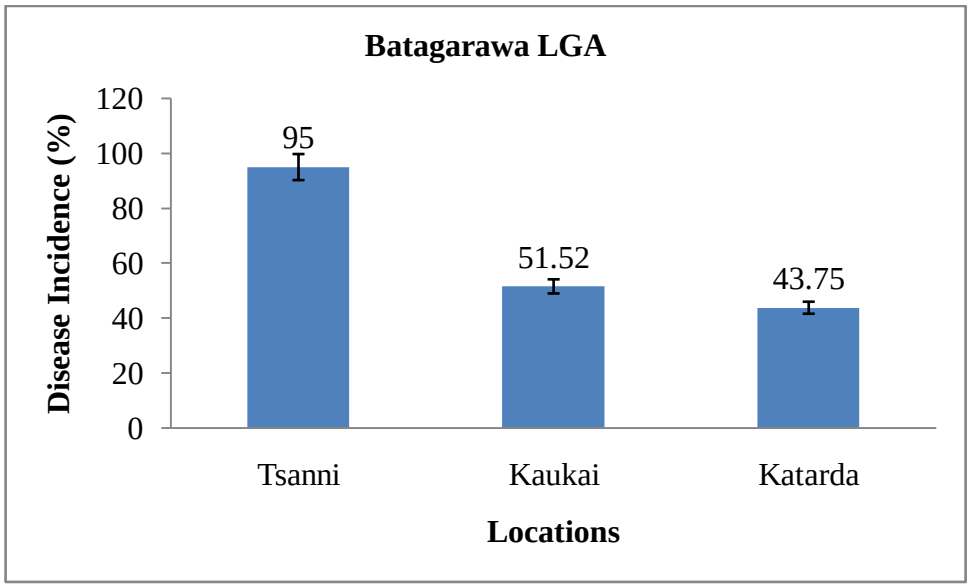


Figure 13: Incidence of *Jatropha mosaic virus* in Batagarawa Local Government Area of Katsina State

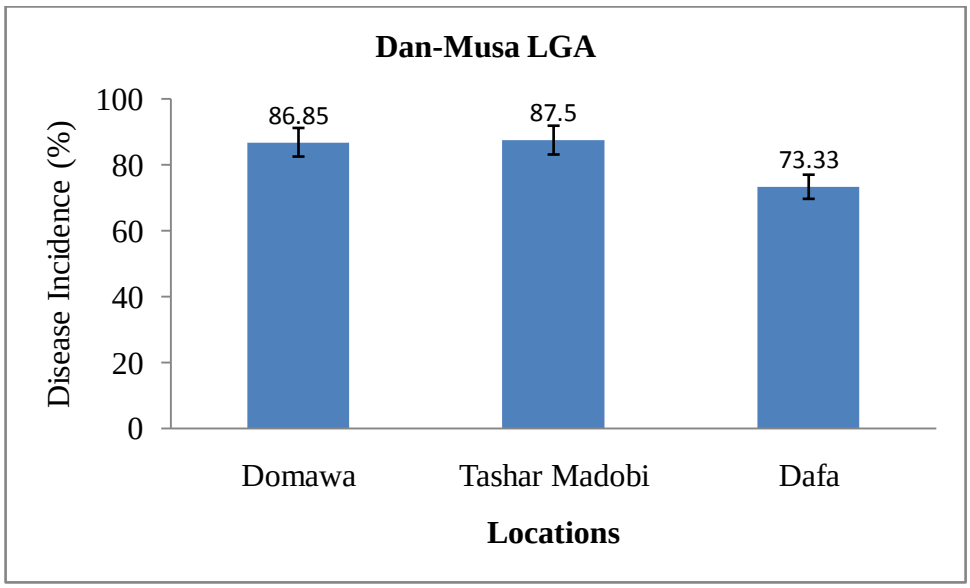


Figure 14: Incidence of *Jatropha mosaic virus* in Dan-Musa Local Government Area of Katsina State

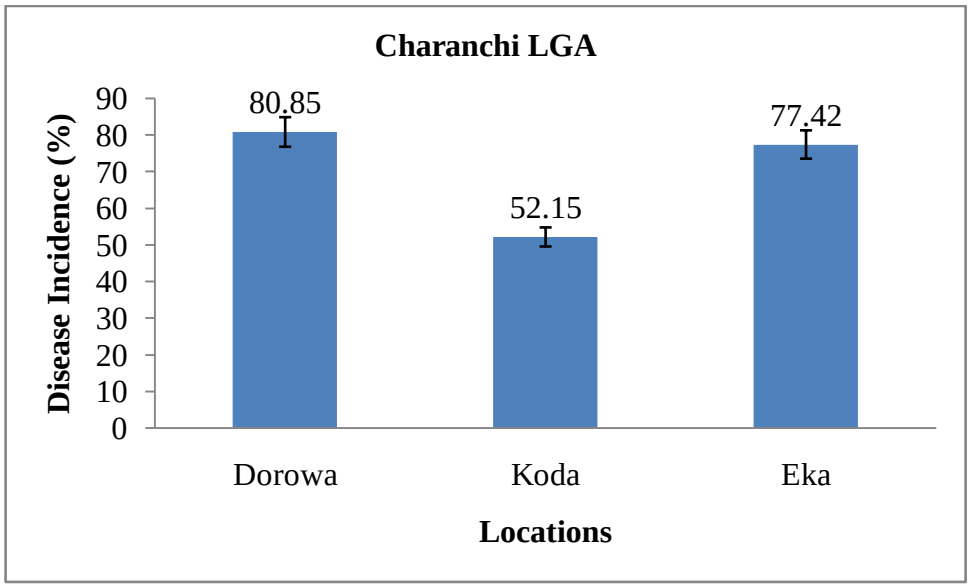


Figure 15: Incidence of *Jatropha mosaic virus* in Charanchi Local Government Area of Katsina State

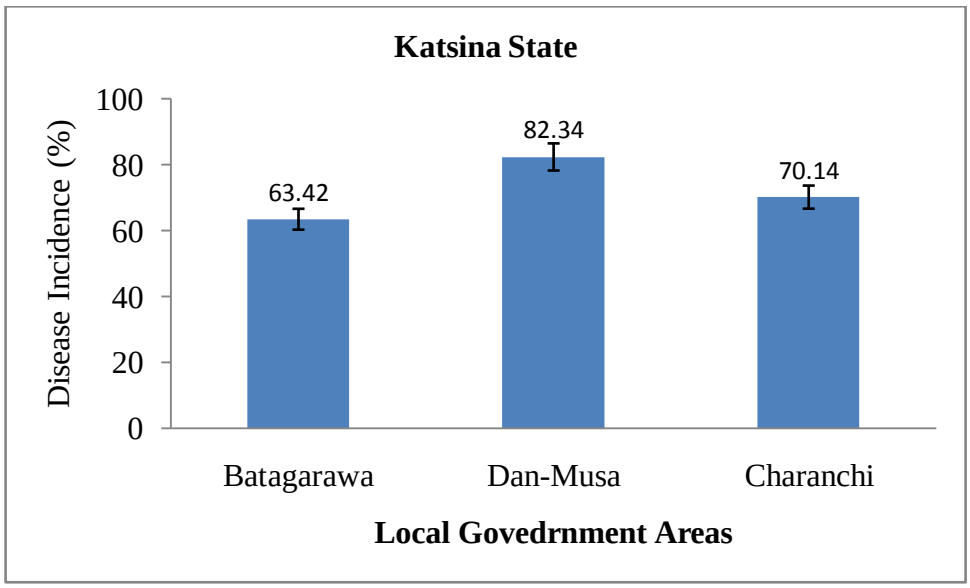


Figure 16: Incidence of *Jatropha mosaic virus* in Katsina State

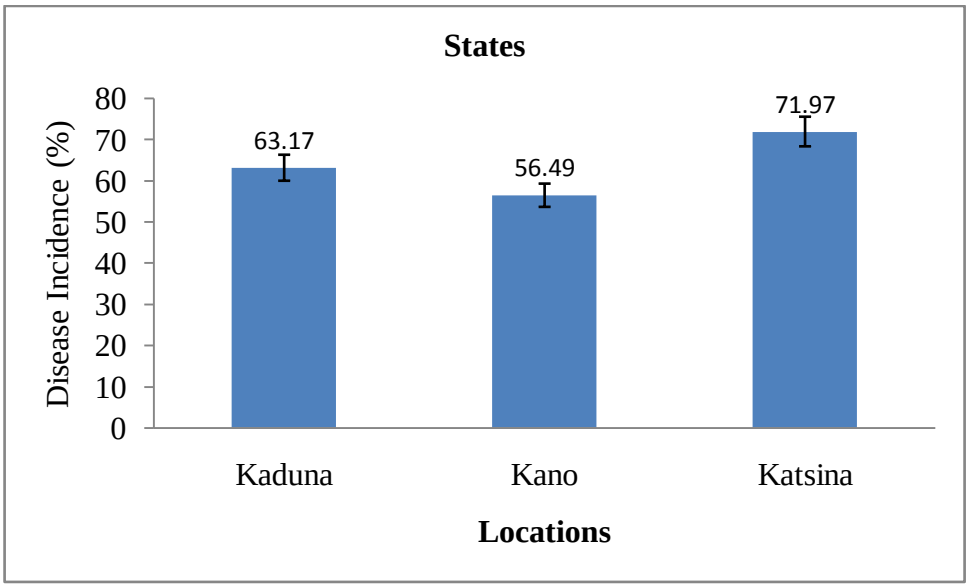


Figure 17: Incidence of *Jatropha mosaic virus* in the three States of Northwestern Nigeria combined

monopartite begomovirus. The results show that subviral β (satellite virus) component of Jatropha mosaic virus was positively identified by PCR. The results also show that geminiviral A and B and sub-viral 1 components were not positively identified by PCR.

Further tests using the JumpStart REDTaq Ready Mix with universal primers **Core AV** (GCCHATRTAYAGRAAGCCMAGRAT)/**Core AC** (GGRTTDGARGCATGHGTACANGCC) and a different set of specific primers **JaFwd1** (CCTTCTYGTCTCCCCTATC) and **JaRev1** (ACCAGGTCAGCACATTTCCA) to amplify the helper virus DNA-A molecule again also had no DNA fragment successfully amplified on samples collected across all locations suggesting the occurrence of beta satellite virus (DNA- β) inthe absence of its helper virus, DNA-A.

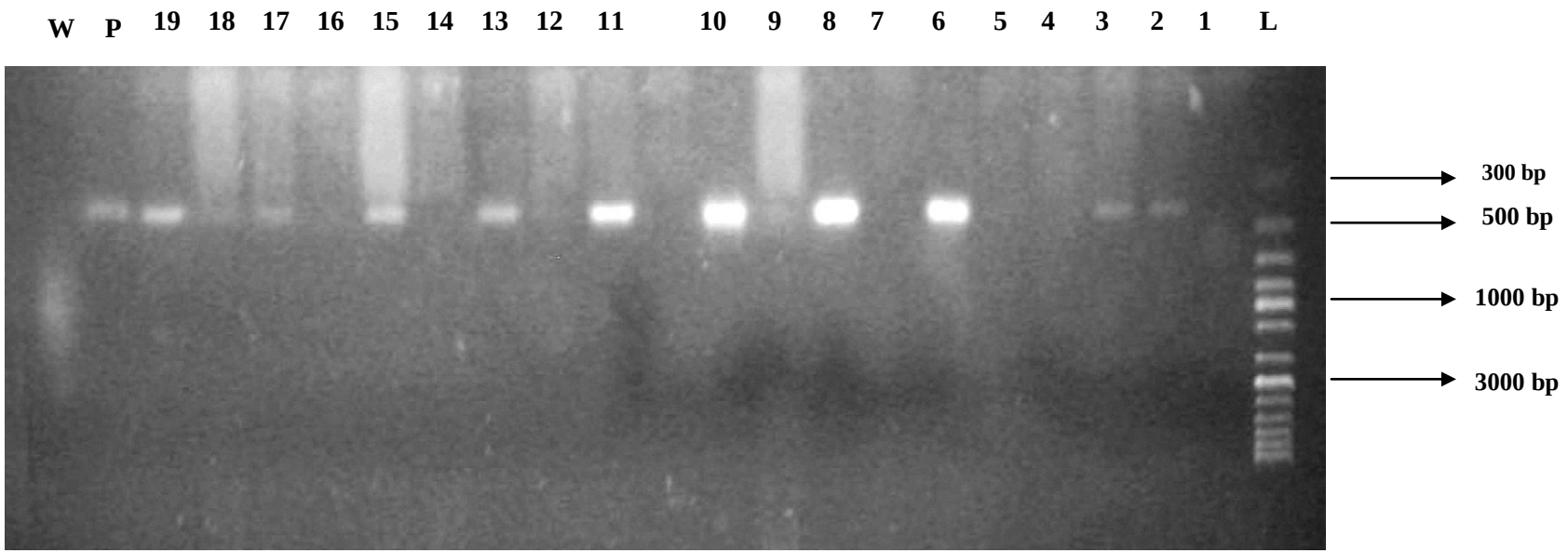


Plate IV: Electrograph of DNA-β PCR products on Agarose gel of NARICT field in Sabon-Gari Local Government Area of Kaduna State.

N2, N3, N6, N8, N10, N12, and N14 – NARICT symptomatic samples
N16, N18, N20 – NARICT asymptomatic samples
L- Molecular size reference ladder (O'GeneRuler 1 kb DNA Ladder), **P**- Positive control (Squash leaf curl virus), **W**- Negative control (water)



Plate V: Electrophoresis of DNA-β PCR products on Agarose gel of IAR field in Sabon-Gari Local Government Area of Kaduna State.

I5, I8, I10 – IAR symptomatic samples
I16, I20 – IAR asymptomatic samples
L- Molecular size reference ladder (O'GeneRuler 1 kb DNA Ladder), **P**- Positive control (Squash leaf curl virus), **W**- Negative control (sterile distilled water)

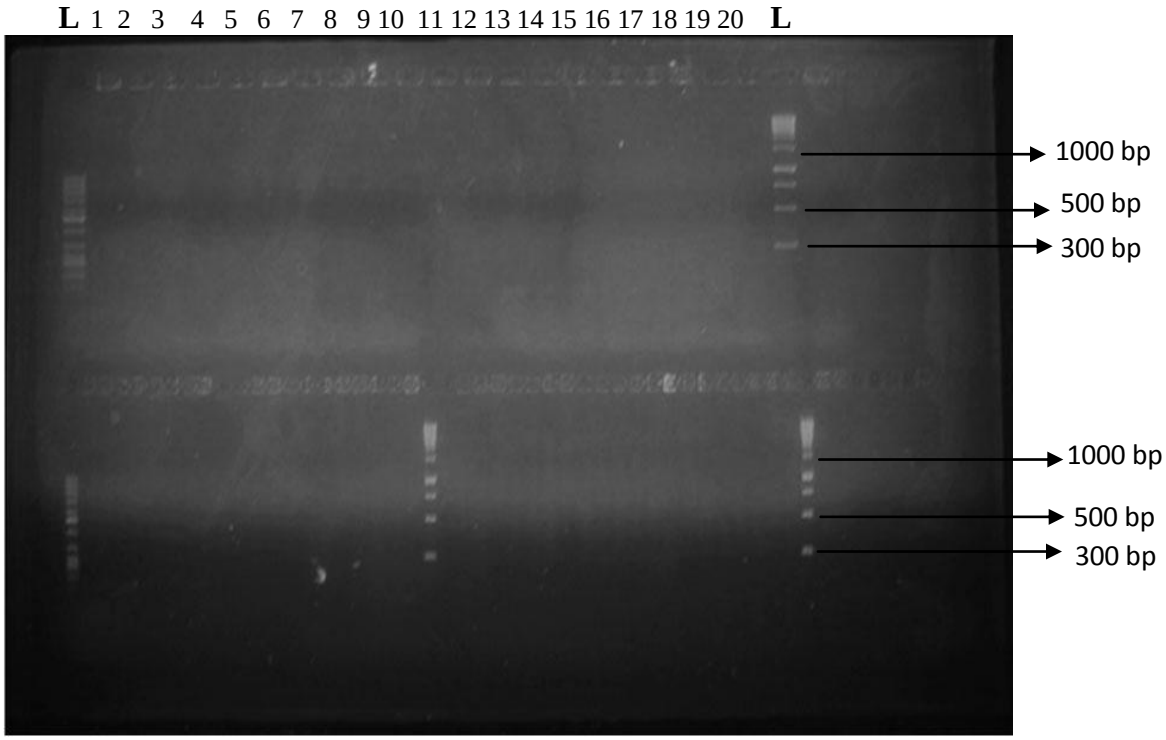


Plate VI: Electrograph of DNA- β PCR products on agarose gel of jatropha fields in Batagarawa Local Government Area of Katsina State.

1 – 20 – Symptomatic and asymptomatic Samples

L – Molecular size reference ladder (O'Generuler 1 kb DNA ladder)

P – Postive control (PCR mastermix + primer)

N- Negative control (Sterile distilled water)

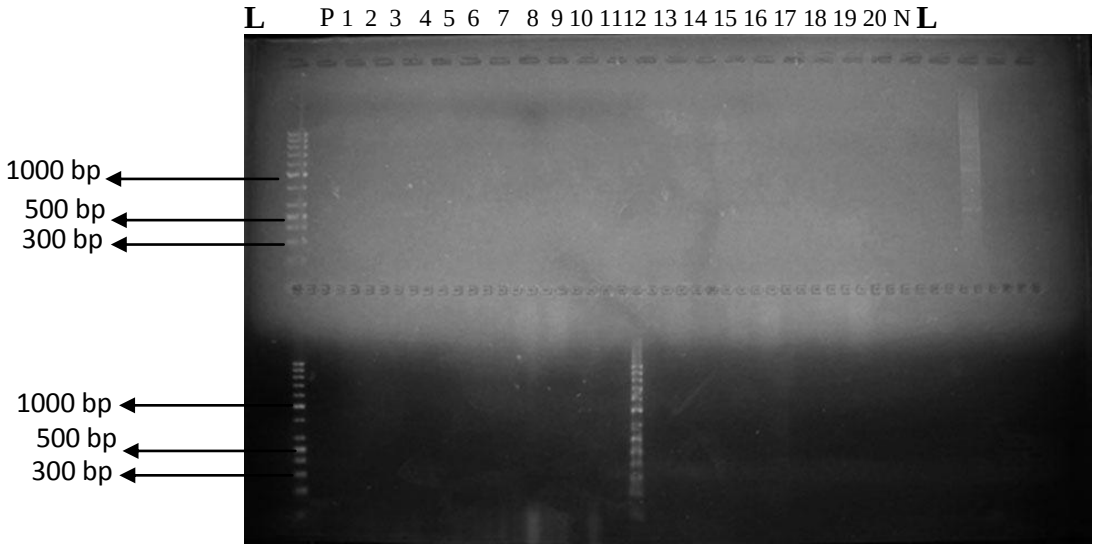


Plate VII: Electrograph of DNA- β PCR products on Agarose gel of jatropha fields in Dan-Musa Local Government Area of Katsina State.

1 – 20 – Symptomatic and asymptomatic Samples

L – Molecular size reference ladder (O'Generuler 1 kb DNA ladder)

P – Postive control (PCR mastermix + primer)

N- Negative control (Sterile distilled water)

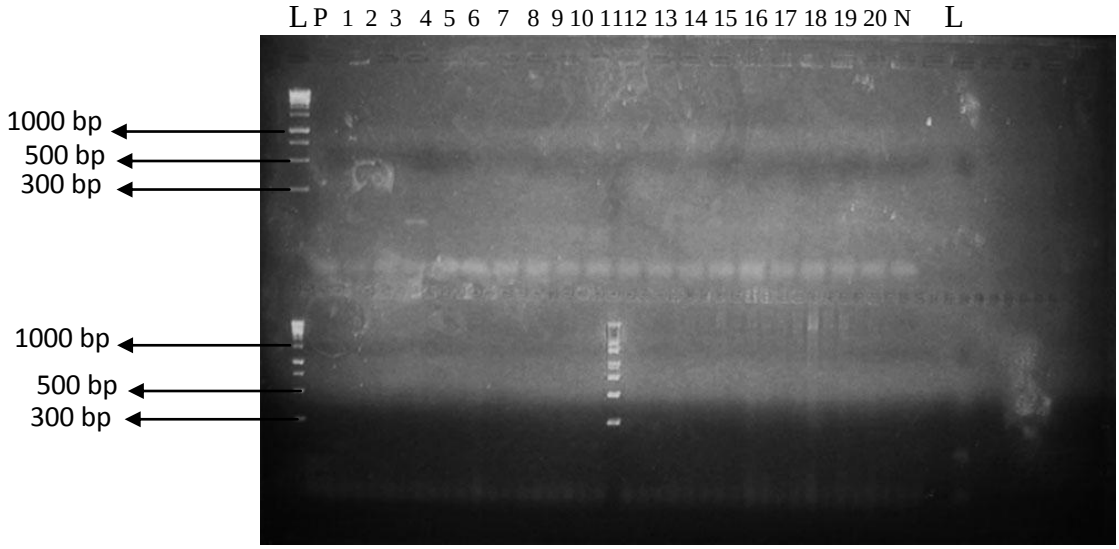


Plate VIII: Electrograph of DNA- β PCR products on Agarose gel of jatropha fields in Charanchi Local Government Area of Katsina State

1 – 20 – Symptomatic and asymptomatic Samples

L – Molecular size reference ladder (O'Generuler 1 kb DNA ladder)

P – Postive control (PCR mastermix + primer)

N- Negative control (Sterile distilled water)

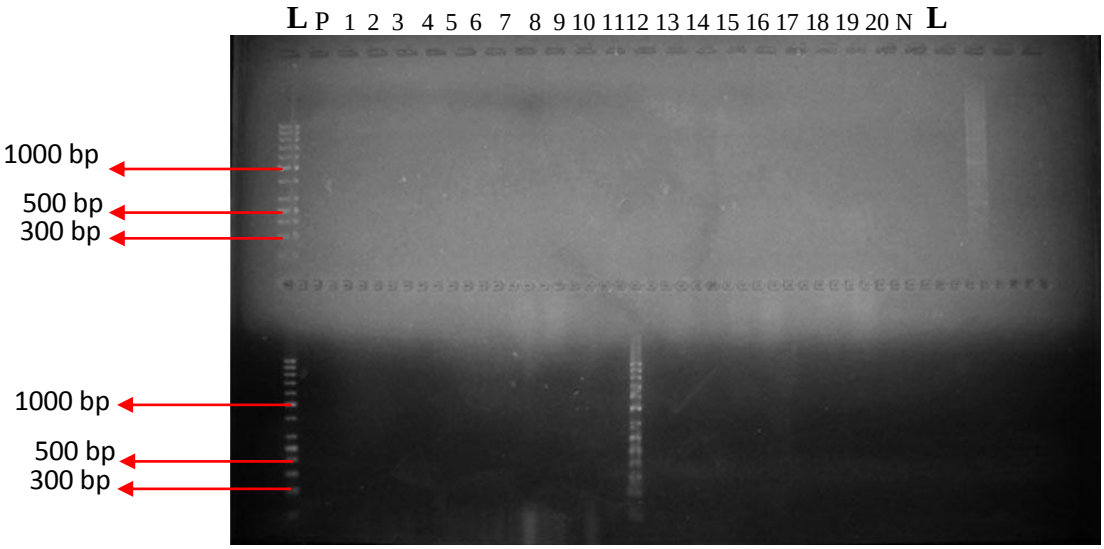


Plate IX: Electrograph of DNA- β PCR products on Agarose gel of jatropha fields in Minjibir Local Government Area of Kano State.

1 – 20 – Symptomatic and asymptomatic Samples

L – Molecular size reference ladder (O'Generuler 1 kb DNA ladder)

P – Postive control (PCR mastermix + primer)

N- Negative control (Sterile distilled water)

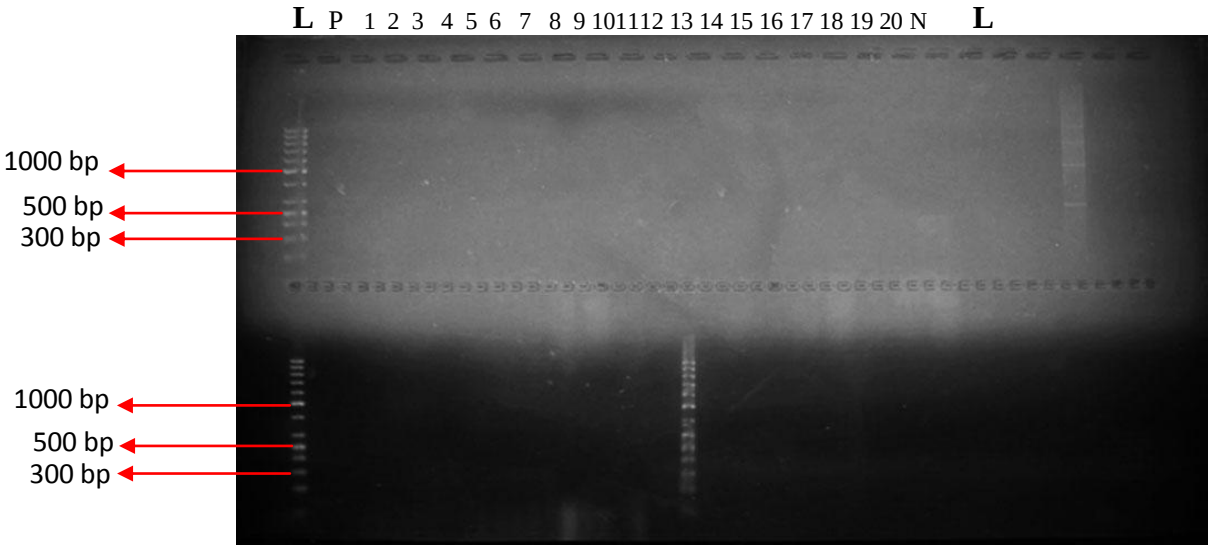


Plate X: Electrograph of DNA- β PCR products on Agarose gel of jatropha fields in Tudun-Wada Local Government Area of Kano State**.**

1 – 20 – Symptomatic and asymptomatic Samples

L – Molecular size reference ladder (O'Generuler 1 kb DNA ladder)

P – Postive control (PCR mastermix + primer)

N- Negative control (Sterile distilled water)

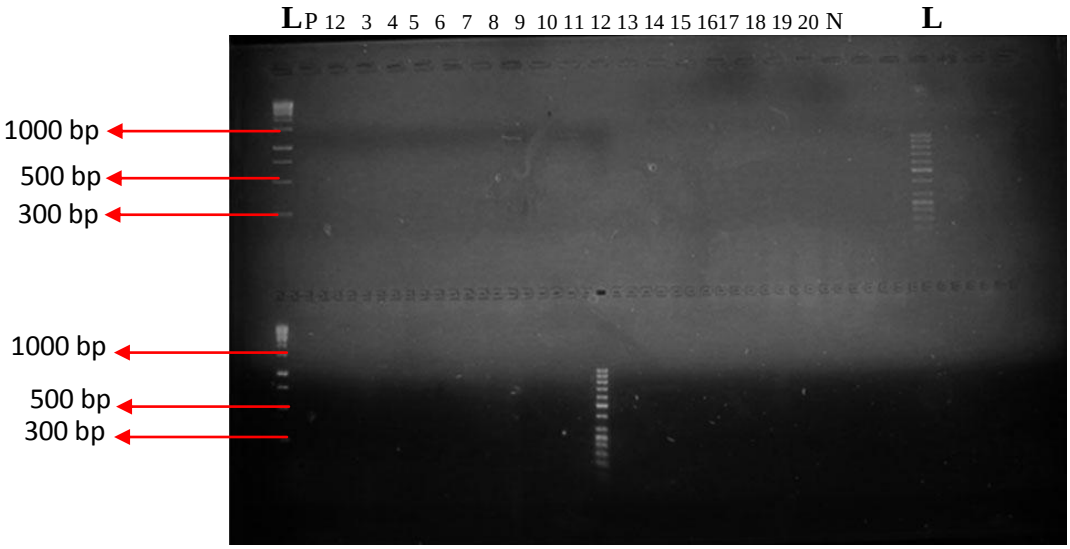


Plate XI: Electrograph of DNA- β PCR products on Agarose gel of jatropha fields in Wudil Local Government Area of Kano State.

1 – 20 – Symptomatic and asymptomatic Samples

L – Molecular size reference ladder (O'Generuler 1 kb DNA ladder)

P – Postive control (PCR mastermix + primer)

N- Negative control (Sterile distilled water)

4.3 Alternate hosts of Jatropha mosaic virus

None of the weed species exhibited peculiar virus disease symptoms. The weeds collected were as follows;*Senna obtusifolia, Sida acuta, Indigotera ciliaris, Piliosligma reticullatum, Senna occidentalis, Ageratum conyzoides, Andropogon gayanus, Lantana camara, Pennisetum pedicellatum, Grewia pubescens, Guiera senegalensis, Scelerocarpus africanum, Monechma ciliatum, Ocimum gratissimum, Capsicum annum, Alium cepa Solanum lycopersicum, Ipomoea batatas and Mangifera indica*. DNA fragment was not successfully amplified using the specific primers on samples collected across all locations.

Further tests using the JumpStart REDTaq Ready Mix and universal primers **Core AV** (GCCHATRTAYAGRAAGCCMAGRAT)/**Core AC** (GGRTTDGARGCATGHGTACANGCC) to amplify DNA-A component also had no DNA fragment successfully amplified on samples collected across all locations.

CHAPTER FIVE

5.0 DISCUSSION

In general, the symptoms observed were mosaic, chlorotic specks on leaves, mottling and blistering of leaves, curling and malformation of leaves, reduction in leaf size and stunting of early infected plants. These symptoms were in conformity with the findings of Bird (1957), Jayanna (2006) and Kashina *et al*. (2013).

Most geminiviruses have been identified using Polymerase Chain Reaction (PCR) which is an extremely sensitive and specific technique (Saiki *et al*., 1988 and Innis *et al*., 1990) for the detection and identification of plant pathogens, and it can be used to investigate the precise questions about the composition and genetic diversity of viruses (Gilbertson *et al*., 1991; Robertson *et al*., 1991). Based on the use of PCR technique, characteristics observed in this study indicates the presence and occurrence of a satellite DNA virus- Beta satellite of JMV conforming to Rojas *et al*., (1993) who reported that geminiviruses are well suited to PCR methods because they replicate via a double-stranded, circular DNA form. The PCR studies led to the detection of a sub-viral component DNA-β suggesting the presence of a monopartite begomovirus.

The PCR technique was employed to detect the geminiviral and sub-viral components of *Jatropha mosaic virus*. The results show that the two geminiviral components DNA-A and B were not successfully detected on all samples tested, so also the sub-viral component DNA-1. This does not conform with the reports of Rojas *et al*., who detected the presence of sub-viral components of a Begomovirus with its helper virus component DNA-A present. Whereas, the

subviral beta satellite component (DNA-β)was successfully detected on some samples of NARICT *Jatropha curcas* field (N2, N3, N6, N8, N10, N12, N14, N16, N18, N20) and IAR *Jatropha curcas* field (I5, I8, I10, I16, I20) from Sabon-Gari Local Government Area of Kaduna State. Since DNA satellite molecules were initially reported to lack sequence identity to their helper viruses and depend on the helper virus for all or some of their functions (gene replication, movement, encapsidation and transmission) results from this study indicates that DNA-A viral component is present and caused infection hence, the symptoms expression but when subjected to molecular assay using PCR, it was not detected. This is in conformation with Tao and Zhou (2004) who reported a beta-satellite *Tomato yellow leaf curl China virus isolate Y10 (TYLCCNV-Y10)* is required for symptom expression in plants but depends on TYLCCNV-Y10 genomic DNA (DNA-A) for replication and encapsidation. They demonstrated previously that DNA-A of *Tomato yellow leaf curl China virus* isolate Y10 (TYLCCNV-Y10) could infect *Nicotiana benthamiana*, *N. glutinosa*, *N. tabacum* Samsun (NN or nn), and *Lycopersicon esculentum* but induced no symptoms. However, inoculation with TYLCCNV-Y10 DNA-A plus Y10 DNA-β induced leaf curl symptoms in these four species (Zhou *et al*., 2003). After repeated PCR tests using a different set of specific primers **JaFwd1** (CCTTCTYGTCTCCCCTATC) and **JaRev1** (ACCAGGTCAGCACATTTCCA) to amplify the DNA-A molecule again, the results were negative also suggesting the occurrence of beta satellite virus (DNA- β) in the absence of its helper virus. This is the first report on detection of a satellite virus of a begomovirus from jatropha crop in Nigeria. The absence of the monopartite genome (DNA-A) can be attributed to it not positively detected but was responsible for typical *Jatropha mosaic virus* disease symptoms. It's possible the DNA was

somewhat degraded and therefore the virus genomes were fragmented and so did not amplify or there was insufficient DNA template for illumina sequencing.

Begomovirus/DNA-β disease complexes induce a variety of host-specific symptoms ranging from a vein yellowing phenotype in ageratum, eupatorium and honeysuckle, that has no obvious adverse effect on plant survival (Wong *et al*., 1993; Saunders *et al*., 2003; Were *et al*., 2005a), to severe leaf curl, chlorosis and stunting in crops such as cotton, tobacco and mungbean, that can have a significant effect on productivity (Harrison *et al*., 1997; Briddon and Markham, 2000; Cui *et al*., 2004b; Rouhibakhsh and Malathi, 2005).This is the first report of infection and expression of disease symptoms by a satellite virus (DNA- β) without its helper virus DNA-A on *Jatropha curcas* in Northwestern Nigeria.

The results of alternate hosts studies of *Jatropha mosaic virus* were negative because none of the amplicons were successfully amplified. This may be due to none of the samples collected showing peculiar begomovirus symptoms and the low concentration, contamination and purity of genomic DNA extracted. Also, most of the weed plants collected did not belong to the same family Euphorbiaceae as Jatropha indicating the absence of the insect vector *Bemisia tabaci* responsible for transmitting the virus.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 SUMMARY

Sample collection was carried out in Kaduna, Katsina and Kano States to determine the incidence of *Jatropha mosaic virus* in Nigeria. Symptomatic and asymptomatic *Jatropha curcas* leaf samples were collected from the field and subjected to Polymerase Chain Reaction (PCR) to detect the presence of geminiviral and sub-viral DNA components.

Results obtained from the study showed that *Jatropha mosaic virus* disease is prevalent in Kaduna, Kano and Katsina States of Northwestern Nigeria and the disease could be positively detected using Polymerase Chain Reaction (PCR). The viral DNA component studies using polymerase chain reaction procedures and primer pairs designed to amplify 0.3 to 0.5 kb fragment of the sub-viral genome revealed that satellite viruses might now be able to cause disease infection and subsequently, symptom expression without their helper virus, although no report on this has been recorded. However, the study did not identify any alternate host growing within and around *Jatropha curcas* fields. There is no clear indication that satellite viruses can cause disease infection in the absence of their helper viruses.

6.2 CONCLUSION

1. *Jatropha mosaic virus* (JMV) was present in all States surveyed with varying incidences. A satellite virus DNA-β was detected in two fields in Sabon-Gari LGA of Kaduna State in the absence of its helper virus DNA-A. This is the first report of a satellite virus DNA-β inducing typical virus symptoms and causing infection on *Jatropha curcas* in Kaduna State.

2. *Jatropha mosaic virus* was not successfully detected in all the weed samples tested across all the fields.

6.3 RECOMMENDATIONS

The following recommendations can be deduced from the results of the current research work:

1. Further studies should be done on *Jatropha curcas* fields and hedges found in other States to have a better knowledge of the distribution of the disease in Northwestern Nigeria and other agro-ecological zones.
2. Further studies should be carried out to ascertain the reason(s) why a satellite virus DNA- β is causing infection and disease symptom expression in the absence of its helper virus, DNA-A.
3. Further study on the movement of the virus particle along the plant should be considered.
4. I recommend that further extensive study on a wider alternative host range of *Jatropha mosaic virus* be considered.

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APPENDIX I

Buffer Recipes

All buffers were constituted in 100 ml.

CTAB Buffer

10 ml 1M Tris, HCl pH 8.0, 28 ml 5 M NaCl, 4 ml of 0.5 M EDTA, 2 g of CTAB (cetyltrimethyl ammonium bromide). Bring total volume to 100 ml with ddH_2O.

TE Buffer

1 ml 1 M Tris, HCl pH 8.0, 0.2 ml 0.5 M EDTA, bring total volume to 100 ml with ddH_2O.

1 M Tris HCl pH 8.0

12.11 g Tris, dissolved in 70 ml of H_2O, pH adjusted to 8.0 by adding concentrated HCl (about 5 ml), bring total volume to 100 ml with ddH_2O.

0.5 M EDTA

18.61 g EDTA, 70 ml H_2O, 1.8 g of NaOH pellets, pH adjusted to 8.0 by adding a few more pellets. Bring total volume to 100 ml with ddH_2O.

5 M NaCl

29.2 g of NaCl, 70 ml H_2O, dissolve and bring volume to 100 ml.

7.5 M Ammonium acetate

57.81 g ammonium acetate, 50 ml of H_2O, bring to 100 ml total volume.

Appendix II: Jatropha cultivation practices adopted by farmers in surveyed fields in Kaduna State

LGA	Location/field	Cropping history (years)	Cropping pattern	Surrounding crops	Spacing (m)	Management practices	Variety
Sabon-gari	I.A.R (samaru)	11	Sole cropping	Non	2 x 2	Weeding, fungicides	Accessions from different parts of Nigeria
	NARICT (Basawa)	8	Sole cropping	*Moringa olifera*	2 x 3	Weeding, fungicides	Indian
	Dogarawa	Non	Border crop	Sugarcane	1 x 1	Non	Unknown
Jema'a	Godogodo	Non	Border crop	Cocoyam	1 x 1	Pesticides	Unknown
	Kafanchan	Non	Border crop	Cocoyam, maize	1 x 1	Pesticides	Unknown
	Kamrum	Non	Border crop	Cocoyam	1 x 1	Non	Unknown
Igabi	Jaji	Non	Border crop	Sweet potato	1 x 1	Weeding, pesticides	Unknown
	Kan gimi	Non	Border crop	Sugar cane, sweet potato	1 x 1	Pesticides, weeding	Unknown
	Gwaraji	Non	Border crop	Tomato, pepper	1 x 1	Weeding, pesticides	Unknown

Appendix III: Jatropha cultivation practices adopted by farmers in surveyed fields in Katsina State

LGA	Location/field	Cropping history (years)	Cropping pattern	Surrounding crops	Spacing (m)	Management practices	Variety
Batagarawa	Tsanni	9	Sole cropping	Mango	5 x 3	Weeding, fungicides	Indian
	Kaukai	Non	Border crop	Sugarcane	1 x 1	Weeding, fungicides	Unknown
	Katarda	Non	Border crop	Sugarcane	1 x 1	Non	Unknown
Dan-Musa	Domawa	Non	Border crop	Groundnut	1 x 1	Pesticides	Unknown
	Tashar Madobi	Non	Border crop	Groundnut, maize	1 x 1	Pesticides	Unknown
	Dafa	Non	Border crop	Maize, groundnut	1 x 1	Non	Unknown
Charanchi	Dorowa	Non	Border crop	Sugarcane, maize	1 x 1	Pesticides	Unknown
	Koda	Non	Border crop	Sugar cane, maize	1 x 1	Pesticides	Unknown
	Eka	Non	Border crop	Maize, pepper	1 x 1	Weeding	Unknown

Appendix IV: Jatropha cultivation practices adopted by farmers in surveyed fields in Kano State

LGA	Location/field	Cropping history (years)	Cropping pattern	Surrounding crops	Spacing (m)	Management practices	Variety
Minjibir	I.A.R (out station)	10	Sole cropping	Non	5 x 7	Non	Matsaya
	Kunya	Non	Border crop	Sorghum, maize	1 x 1	Pesticides	Unknown
	Gurjiya	Non	Border crop	Soybean	1 x 1	Pesticides	Unknown
Wudil	Gano	Non	Border crop	Soybean, maize	1 x 1	Pesticides, weeding	Unknown
	Kafin Mallamai	Non	Border crop	Sorghum, maize	1 x 1	Pesticides, weeding	Unknown
	Kumfada	Non	Border crop	Sorghum, soybean	1 x 1	Non	Unknown
Tudun-wada	Gazobir Tsohurwa	Non	Border crop	Sorghum, millet	1 x 1	Weeding, pesticides	Unknown
	Ungwar Ama	Non	Border crop	Millet, maize	1 x 1	Pesticides, weeding	Unknown
	Marmara	Non	Border crop	Maize, sugarcane	1 x 1	Weeding, pesticides	Unknown