SURVEY AND MOLECULAR DETECTION OF BANANA (*MUSA ACCUMINATA* COLLA) VIRUSES IN SELECTED LOCAL GOVERNMENT AREAS OF KADUNA STATE, NIGERIA

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A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA
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NIGERIA

DECEMBER, 2014

DECLARATION

I declare that the work in this thesis entitled ‘‘SURVEY AND MOLECULAR DETECTION OF BANANA (*MUSA ACCUMINATA* COLLA) VIRUSES IN SELECTED LOCAL GOVERNMENT AREAS OF KADUNA STATE AND THE ROLE OF WEEDS AND INSECTS IN THE TRANSMISSION OF THE VIRUSES’’ has been carried out by me in the Department of Biological Sciences. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any other Institution.

Aisha Muhammed ZONGOMA _____ _____

Signature Date

CERTIFICATION

This thesis entitled ‘‘SURVEY AND MOLECULAR DETECTION OF BANANA (*MUSA ACCUMINATA* COLLA) VIRUSES IN SELECTED LOCAL GOVERNMENT AREAS OF KADUNA STATE, NIGERIA’’ by Aisha Muhammed ZONGOMA meets the regulations governing the award of the degree of M.Sc. Botany of the Ahmadu Bello University, and is approved for its contribution to the knowledge and literary presentation.

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DEDICATION

I dedicate this thesis to Almighty Allah and my father: Late Mal Muhammed Zongoma.

ABSTRACT

Survey for banana viruses was conducted in three local government areas (Igabi, Zaria and Chukun) of Kaduna state between February and July 2013. Structured questionnaire was administered to farmers to ascertain farming system, pests and diseases associated with banana cultivation. The farmers were aware of both Cucumber mosaic virus (CMV) and Banana streak virus (BSV) symptoms however they associated it to water stress, overcrowding and insufficient manure rather than viral disease. Both asymptomatic and symptomatic banana leaves, weeds as well as insects associated with banana plants were sampled. Polymerase chain reaction (PCR) was used for the detection of Banana bunchy top virus (BBTV) and BSV while reverse transcriptase polymerase chain reaction (RT-PCR) was used for the detection of CMV and Potyvirus (RNA viruses). Based on the analysis, there were amplification in Banana samples for BSV and CMV while BBTV and Banana bract mosaic virus (BBrMV) were not detected. Symptomatic banana leave samples were negative for BSV using BSV Onne primer while some asymptomatic banana leaves were positive for BSV using BSV Onne primer suggesting another isolate of BSV. There was significant difference ($p<0.05$) for symptom severity and PCR score indicating that the severity of the disease and PCR score depends on locations and there was variation in BSV symptom expression with these locations. Weeds samples showed amplification for BSV, CMV and BBrMV. The 5 weed samples positive for Potyviruses were also positive for BSV indicating that the samples have multiple infection. *Commelina diffusa* collected from different locations were positive for potyvirus and BSV indicating that it is a good reservoir host for the virus. None of the banana viruses were detected in mealybugs and aphids sampled.

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LIST OF ABBREVIATIONS

ACP Antigen coated plate

BBrMV Banana bract mosaic virus

BBTV Banana bunchy top virus

BSCavV Banana streak virus Cavendish isolate

BSGfV Banana streak virus Gold finger isolate

BSMysV Banana streak virus Mysore isolate

BSV Banana streak virus

cDNA Complementary deoxyribonucleic acid (normally synthesized from RNA)

CMV Cucumber mosaic virus

CTAB Cetyltrimethylammonium bromide

dNTPs Deoxynucleoside Triphosphates

ELISA Enzyme linked immuno-sorbent assay

PBS Phosphate buffered saline

PVP Polyvinyl pyrolidone

RT Reverse transcriptase

SAS Statistical Analysis System

TAE Tris Acetate EDTA

TNA Total nucleic acids

CHAPTER ONE

1.0 INTRODUCTION

1.1 Preamble

Banana belongs to the family Musaceae of which includes plantains and ornamental bananas. Banana and plantain originated from Southeast Asia and surrounding tropical and subtropical regions (Ploetz *et al*., 2007). The crop is grown across different agro-ecological conditions ranging from lowland to highland about 1500m above sea level. Banana and plantain rank fourth in the world after rice, wheat and maize in terms of financial value (Treitler, 2006). Banana is one of the most important crop in international trade for earning foreign exchange in many African countries. According to UNCTAD (2012), over 105 million metric tonnes of banana are produced yearly. Approximately 98% of world production is grown in developing countries. More than 73% of total banana production is contributed by ten major banana-producing countries: India, China, Philippine, Equador, Brazil, Indonesia, Tanzania, Guatamala, Angola and Mexico. India, Ecuador, Brazil and China alone produce half of total banana output. India leads the world in banana production with annual output of about 14.2 million tonnes. Africa is a secondary centre of diversity (Ploetz *et al*., 2007). Uganda ranks second in the world in banana production with an annual output of about 10.5 million tonnes (Kubiriba *et al*., 2013). Uganda is the largest producer of Banana in Africa followed by Rwanda, Cameroon, Nigeria and Ghana. Nigeria accounts for 5% of the world production (Faturoti *et al*., 2008). Plantain is one of the primary commodities for investment across the south south zone in Nigeria, and it is known to be centre of plantain and banana diversification.

Banana along with plantains are good sources of dietary carbohydrates; vitamins C and B6 and minerals such as phosphorus, calcium, and potassium, but are fat free and low in proteins. It has several medicinal properties (Dheepa and Paranjothi, 2010). Fruits are good for the treatment of gastric ulcer, diarrhoea, cancer and heart diseases. Production of food staples provide the rural poorest with most of their work income (Kedir, 2003).

Biotic stresses induced by fungi, bacteria, nematodes, viruses and insect pests affect the productivity of banana in many countries where the crop is grown. Among the biotic constraints, viruses cause huge economic loss to the crop (Dheepa and Paranjothi, 2010). Five viruses have been reported to infect banana worldwide (Anonymous, 2009). These are: Cucumber mosaic virus (CMV) genus *Cucumovirus*, Banana bunchy top virus (BBTV) genus *Nanovirus*, Banana streak virus (BSV) genus *Badnavirus*, Banana mild mosaic virus (BanMMV) and Banana bract mosaic virus (BBrMV) genus *Potyvirus*. However in Sub-Saharan Africa, BSV, BBTV, BBrMV and CMV are the most important viruses that have been reported (Kubiriba and Tushemereirwe, 1999). Banana viruses are transmitted through vegetative planting material (suckers/sets) and vectors (mealybugs and aphids). Among the viruses of banana, banana bunchy top disease is the most serious disease of Banana with yield loss of up to 100% as reported in Pakistan. Estimated yield losses of between 7-90% have been attributed to banana streak virus (Harper *et. al*., 2004; Davis *et al*., 2000; Daniells *et. al*., 2001). Bunch weight reduction by CMV of between 45% and 62% has been reported in Kerala, India (Estelitta *et al*., 1996).

Even though there is long history of banana cultivation in the southern (rainforest) region of Nigeria, however, certain percentage is cultivated in the northern guinea savannah zone where Kaduna state lies. Most of the researches on viruses affecting

Banana were reported from southern part of the country and little or no documentation of banana viruses in the northern part of the country. A number of techniques have been used for the detection of banana viruses of which the molecular method is most currently used.

1.2 Statement of Problem

Banana virus diseases are among the most important diseases of banana causing high economic loss (Dheepa and Paranjothi, 2010). The viruses are transmitted by vectors (i.e. aphids and mealybugs) and in vegetative planting material i.e. sucker. Hence, once the mother plants used for mass multiplication are not diagnosed, the viruses can easily pass through to the progenies.

Despite the suitability of Kaduna state for the cultivation of banana, there is little or no information on occurrence and identity of viral diseases affecting banana in the area. Also, there is little information on the role of weeds growing around banana fields as reservoir hosts as well as perception of farmers about the disease.

1.3 Justification

Banana (*Musa* spp.) is one of the most important subsistence crop in the world. It is widely grown in the tropics and subtropics in about 150 countries (Benson, 2013) in all types of agricultural systems, from small, mixed subsistence gardens to large multinational commercial monocultures. In many African countries like Ghana, Rwanda and Cameroon, the crop serves as the staple food for the population or the cornerstone of the country's economy. Apart from consumption as dessert, banana is used for the treatment of a number of diseases (Dheepa and Paranjothi, 2010).

Banana is produced on a large-scale in the humid rain forest production zone as well as in the sub-humid areas of Nigeria. Kaduna state lies at an altitude of 614-704 m above sea level with relative humidity of 60-80% which makes it suitable for banana cultivation especially the southern part of the state. Banana is cultivated either as orchards or as hedge around tomato and sugarcane farms. About 49% of the farming households in Nigeria produce banana as their main crop. Impact studies in Nigeria reported that farmers were earning net incomes of $200 per season from the sale of suckers, in addition to around $8,000 per hectare from fruit sales (*www.new-ag.info/09/01/dl/focuson.rtf*). Banana in Nigeria is consumed as dessert, porridge or pounded. It is used traditionally for the treatment of hypertension, cataract and the combination of unripe dried banana and plantain is used locally in Nigeria for the permanent treatment of stomach ulcer.

Viral diseases pose a major challenge to banana production worldwide (Anonymous, 2009). The main viral diseases include: Banana streak badnavirus (BSV), Banana bract Potyvirus (BBrMV), Cucumber mosaic cucumovirus (CMV) and Banana bunchy top nanovirus (BBTV). The Identification and detection of viral diseases affecting banana have been reported in Asia and the Pacific as well as from some African countries like Ghana, Egypt, Uganda and Nigeria (southern and western) using both serological and molecular technique. For example, BSV was detected using TAS-ELISA, PCR and Rolling circle amplification (Su, 2009; Manoranjitham *et al.,* 2012; Wambulwa *et al*., 2012). Agindotan *et al.,* (2006) detected BSV using both ELISA and PCR technique. Also, BBTV was detected using PCR and TAS EISA (Su, 2009, El-Dougdoug *et al*., 2011). CMV was been detected using both TAS-ELISA and PCR technique (Agindotan *et al.,* 2006, Su 2009). BBrMV was also detected using PCR (Su, 2009)

In Nigeria, most of the work done on detection and identification of banana viruses were from southern and western part of the country. For example, Kubiriba and Tushmereirwe (1999) reported the occurrence of BSV, BBTV and CMV from southern Nigeria. Similarly, Agindotan *et al*. (2006) detected BSV in western Nigeria using PCR and ELISA techniques. Despite the above information, little or no work has been done on detection of viruses infecting banana in northern part of the country. Kaduna state has a long history of banana cultivation and the crop contributes to the income of the peasant farmers. Banana cultivation is receiving much attention both locally and by commercial farmers. Hence, there is the need to isolate and identify the viruses affecting banana in the selected area were the crop is growing using molecular technique.

1.4 Aim and Objectives

The aim of the study was to identify viruses infecting banana crops as well as the role of weeds and insects around banana farms in selected local government areas of Kaduna State Northern guinea savanna zone

The objectives of the study include:

1. Detection of the problems associated with banana cultivation in the study areas.

2. Elucidate the incidence of banana viral diseases in the areas under study using symptoms observed in field and molecular technique.

3. Assessment of weeds growing within and around banana field as reservoir host of the viruses using molecular technique.

4. Detection of banana viruses in insects found associated with banana plants in banana fields using molecular technique.

1.5 Hypotheses

1. There are no problems affecting banana cultivation in the area under study.
2. There is no significant difference in the incidence of banana viral diseases in the areas under study.
3. Weeds found within and around banana field do not serve as reservoir host of the viruses infecting banana.
4. Insects around the banana field do not serve as reservoir host of the disease.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Origin of Banana

The family Musaceae is composed of the genera *Musa*, *Ensete* and *Musella*. De Langhe *et al.* (2009) reported that all edible banana fruits are produced by plants belonging to the genus *Musa*. Banana evolved in the humid tropical regions of South East Asia with India as one of its centres of origin. Modern edible varieties have evolved from the two species: *Musa acuminata* and *Musa balbisiana* and their natural hybrids, originally found in the rain forests of South East Asia. *Acuminata* means long-pointed or tapering, not referring to the fruit, but to the flowers giving birth to the fruit (*Peggy*, 2006). During the seventh century AD its cultivation spread to Egypt and other parts of Africa. Antonius Musa was the personal physician to Roman emperor Octavius Augustus, and it was he who was credited for promoting cultivation of the exotic African fruit from 63 to 14 B.C. (Peggy 2006) . The name banana is derived from its Guinean name 'banema' (Chandramita, 2011).

2.2 Botany of Banana

The banana plant has a cylindrical, succulent, pseudo stem, with a cylindrical leaf petiole sheath reaching a height of 6-7.5 m and arising from fleshy rhizomes or corm. Suckers spring up around the main plant. The oldest sucker replaces the main plant when it fruits and dies, and this process of succession continues indefinitely. Tender, smooth, oblong or elliptic, flesh-stalled leaves numbering 4 to 15, are arranged spirally, and can be up to 2.7 m long and 60 cm wide. The inflorescence, a transformed wing point, is a terminal spike, shooting out from the heart in the tip of the stem (Mnyanwezi, 2014).

Contrary to popular belief, banana plants are not trees but giant herbs, which reach their full height of between 3 to 6 meters after only a year. Every banana blossom develops into a fruit, which is ripe enough for consumption after about three or four months. After producing fruit, the plants' stems die off, and are replaced by new growth (http://www.rainforest-alliance.org). Banana stems have an average of 150 "fingers" and weigh nearly 45 kg. The trunks of banana plants are not woody but composed of sheets of overlapping leaves wrapped tightly around one another, a design feature that enables them to conserve water. Because banana plants are approximately 93 percent water, even moderate winds can knock them down and destroy entire plantations. Banana is normally multiplied vegetatively through suckers. The development of the plant can be divided into three periods: vegetative, flowering and yield formation. The time from planting to shooting (vegetative) is about 7 to 9 months, but with lower temperatures at higher altitudes or in the subtropics, the period can be up to 18 months. The time from shooting to harvest (flowering and yield formation) is about 90 days. In tropical lowlands the time to harvest of the next ratoon crop is about 6 months. The average life of a commercial plantation can be from 3 to 20 years. Some varieties are replanted after each harvest (FAOSTAT, 2013).

2.3 Habitat

Bananas are indigenous to the tropical areas of India, Southeast Asia and northern Australia, and were brought to South America by the Portuguese in the early 16th century. Today, banana plants grow in the humid, tropical regions of Central and South America, Africa, and Southeast Asia where there are high temperatures and rainfall. Modern agricultural technologies also enable people to cultivate banana plants in non-tropical regions such as California in the United States (www.rainforest-

alliance.org). At present banana is being cultivated throughout the warm tropical regions of the world between 30^0 N and 30^0 S of the equator.

2.4 Agro-climatic Requirements for Cultivation of Banana

Banana, basically a tropical crop, grows well in a temperature range of 15 ºC – 35 ºC with relative humidity of 75-85 %. It prefers tropical humid lowlands and is grown from the sea level to an elevation of 2000 m. In India, it is cultivated in climate ranging from humid tropical to dry mild subtropics through selection of appropriate varieties. Chilling injury occurs at temperature below 12 ºC. A humidity of at least 60 percent or more is preferable. Strong winds, greater than 4 m/sec, are major cause of crop loss due to the pseudostems being blown down. Under high wind conditions windbreaks are desirable (FAOSTAT, 2013). Four months of monsoon (June to September) with an average 650-750 mm rainfall are very important for vigorous vegetative growth of banana. At higher altitudes, banana cultivation is restricted to a few varieties like 'Hill banana" (htt://nhb.gov.in/model-project reports/Horticulture%20Crops%5Cbanana%Banana1.htm).

Bananas can be grown in a wide range of soils as long as there is good drainage and adequate fertility. They can tolerate short periods of flooding but do require good soil aeration (Mnyanwezi, 2014). Deep, rich loamy soil with pH between 6.5- 7.5 is most preferred for banana cultivation. Saline solid, calcareous soils are not suitable for banana cultivation. Fertilizer requirements are 200 to 400 kg/ha N, 45 to 60 kg/ha P and 240 to 480 kg/ha K per year (FAOSTAT, 2013).

2.5 World Production

Plantains and bananas represent the world's second largest fruit crop after citrus with an annual production of 129,906,098 million tonnes (Benson, 2013) and grown in nearly 150 countries. Most bananas are planted for sale in local markets or self-

consumption, and only a fraction of all bananas produced are sold in the world market (FAO, 2003). According to FAOSTAT (2013) total world production of banana is about 68.6 million tonnes of fresh fruit. World production of bananas increased by 175% over the last 30 years to 102 million tonnes in 2010 (http://www.cgiar.org/our-research/crop-factsheets/bananas/). India is the largest producer in the world with almost 30 million tonnes, whilst Latin American countries dominate the international banana trade. The ten major world producers of banana are: **India, which produces (29.6 billion kg) followed by** China (10.7 billion kg), Philippines (9.1 billion kg), Equador (7.4 billion kg), Brazil (7.3 billion kg), Indonesia (6.1 billion kg), Tanzania (3.1 billion kg), Guatamala (2.7 billion kg), Angola (2.6 billion kg) and Mexico (1.5 billion kg) (www.seriousrankings.com). East Africa is the largest banana-producing and consuming region in Africa with Uganda being the leading producer, with a total production of about 10.5 million tons. In some African countries such as Uganda the daily consumption of banana may exceed 1.6 kilogram per person, which is the highest in the world (IITA, 2008). Uganda is the largest producer of banana and plantain in sub-saharan Africa (SSA), followed by Rwanda, Ghana, Nigeria, and Cameroon (IITA, 2012). Bananas are very important sources of food with an annual production of more than 130 million tonnes. It has been established that the fruit in sub-Saharan Africa supplies the most important contribution to healthy food of more than 100 million people.

Banana production in Africa has doubled over the last 30 years (10 million metric tons) while in Asia it has quadrupled (62 million metric tons). Total global production ranks fourth after maize, rice and wheat. In the East African highlands, consumption may be as high as 1 kilogram per person per day. Of the numerous edible varieties, the East African Highland Banana (EAHB) accounts for 17 % of the types of Musa

grown worldwide and plantain accounts for another 19 %. There are 120 EAHB varieties in Uganda alone that are not found anywhere else in the world. About 87 % of all the bananas grown worldwide are produced by small-scale farmers for local consumption as a food security crop, and for local markets than for international trade (IITA, 2008). Only 15 % of global banana and plantain production is involved in international trade while most production is consumed domestically (http://www.cgiar.org/our-research/crop-factsheets/bananas/).

Approximately 13 % of worldwide banana production is destined for the export market. The banana fruit is extremely important as an export commodity especially in Latin America and Caribbean, which contribute over 83% of the total banana in the international market. The banana export industry is also the backbone of the economies of many Caribbean countries, and the crop plays a vital role in the social and political fabrics of the islands. In Africa, only five countries namely; Côte d'Ivoire, Cameroon, Somalia, Ghana, and Cape Verde, export approximately 427,000 tons of banana and plantain. There are more than 500 banana varieties in the world, but the Cavendish is the most exported banana cultivar. The banana's ability to produce fruits all year round makes it an important food security crop and cash crop in the tropics (IITA, 2008).

In Africa, bananas (*Musa* spp.) are staple food crops and sources of income that are cultivated as backyard plantings, mixed-crop plantings and in commercial plantations (Viljoen, 2010) Cultivation of bananas in Africa consists essentially of three production systems (Karamura *et al.,* 1999). In the first production system, banana plants are cultivated in combination with traditional food crops on smallholdings of <1 ha. These production areas are often distant from urban centres or homesteads in

rural areas. More recently, banana production moved from distant production centres to fields close to the homestead in rural areas, or to backyards in urban areas. This production system became popular because of a reduction in suitable forestland, problems with transporting heavy bunches over long distances and theft in distant fields. Thirdly, bananas are produced under intensive monoculture systems for the lucrative urban and export markets, principally by the educated class, civil servants and business people with the use of fertilizers and pesticides.

Although some diploid bananas are still grown today, the most widespread and economically important bananas are the triploids, while tetraploids are very rare in nature or they are artificially bred (UNCST, 2007). Banana cultivation in sub-Saharan Africa can roughly be divided into two main production regions: In Eastern and Central Africa banana plantings consists mainly of the East African highland bananas (EAHB) (AAA-EA genome), with a limited amount of ABB genome cooking, beer bananas and plantains (AAB genome). In Western Africa, more than 70 % of bananas produced are plantains, with dessert bananas and ABB cooking bananas making up the rest (Frison and Sharrock, 1998). Less than 3% of bananas produced in sub-Saharan Africa are exported (Viljoen, 2010).

2.6 Economic Importance of Banana

The all year round fruiting habit of bananas puts the crop in a superior position in bridging the 'hunger gap' between crop harvests (UNCST, 2007). Banana leaves are used worldwide as cooking materials, plates, umbrellas, seat pads for benches, fishing lines, clothing fabric and soles for inexpensive shoes. They can be very effective for treatment of patients suffering from anaemia, blood pressure, constipation, depression, heartburn, stress, ulcers in the intestine and are also beneficial in

enhancing brain power (Chandramita, 2011). A person should eat at least 5 portions of fruit and vegetables every day where one whole banana fruit is equivalent to one portion just as two tomatoes and or half cucumber (UNCST, 2007). Banana is a very popular fruit due to its low price and high nutritive value. It is consumed in fresh or cooked form both as ripe and raw fruit. The pulp of a ripe banana is essentially a sugar-rich, easily-digested food. The cooked banana is nutritionally similar to potato. It consists of about 70% water, 27% carbohydrate, 1.2% protein and 0.3% fat. In enegy terms, each gram provides one calorie. The fruit is considered a good source of vitamins A, B1, B2 and C (http://www.cgiar.org/our-research/crop-factsheets/bananas/). It is also a good source of potassium, phosphorus, calcium and magnesium. The fruit is easy to digest, free from fat and cholesterol. Banana powder is used as the first baby food. It helps in reducing risk of heart diseases when used regularly and is recommended for patients suffering from arthritis, gastroenteritis and kidney disorders.

Processed products, such as chips, banana puree, jam, jelly, juice and wine can be made from the fruit. The tender pseudostem, which bears the inflorescence is used as vegetable. Plantains or cooking bananas are rich in starch and have a chemical composition similar to that of potato. Banana fibre is used to make items like bags, pots and wall hangers. Rope and good quality paper can be prepared from banana waste. Banana leaves are used as healthy and hygienic eating plates (htt://nhb.gov.in/model-project reports/Horticulture%20Crops%5Cbanana%Banana1.htm)

2.7 Banana Allergenicity

Banana allergy can take two different forms: one associated with allergy to tree pollen, such as birch, and another type associated with latex allergy. People with birch-pollen allergy can develop symptoms either immediately or up to one hour after eating fresh banana or a banana-containing food. Symptoms comprise local reactions in the mouth and throat with itching and inflammation called oral allergy syndrome (OAS). Others develop banana allergy because of the similarity between the allergens in banana and natural rubber latex a condition known as the latex fruit syndrome. Symptoms developed by these individuals comprise generalized abdominal pain, vomiting and sometimes life-threatening symptoms. These individuals often develop adverse reactions to chestnut, avocado, mango and kiwi. The class I chitinase in these fruits, which belong to a family of pathogenesis-related proteins (PR-3) has been reported to be an important cross-reactive allergen for latex-sensitized people (Diaz-Perales *et al.,* 2003). Allergenicity to bananas increases with ripeness.

2.8 Problems Affecting Banana Cultivation

Over the years a myriad of problems tend to militate against the production of this crop especially in the tropics. Some of these problems are weeds. Robinson, (1996) reported that weeds are a major constraint in the production of this crop for subsistence farmers. In West Africa, weed growth is very prolific and lack of effective weed control is a key factor that reduces yield and this leads to overall yield decline. Other limiting factor for plantain and banana production is the occurrence of pest and diseases that reduces yield drastically by more than 30 to 50 %. According to Shaibu *et al.* (2012), there are some yield reduction constraints such as pests, diseases and weed infestation that limit expansion of banana in that part of the country.

2.9 Management of Weeds in Banana Cultivation

Currently, most researchers are particularly concerned with identifying management techniques that could reduce weed without paying any attention to the economics of such technique. Anderson (1996) reported that weeds in plantain can be controlled through mulching, use of herbicides and manually. According to Obiefuna (1989), it is also feasible to control weeds in plantain biologically using 'Egusi' melon (*Colocynthis citrullus*). Obiefuna (1989) observed that intercropping plantain with 5000 melon seeds per hectare suppressed weed growth and significantly increased plantain yield. At 2500 melon seeds per hectare, weed control was inadequate and at 10,000 melon seeds per hectare, plantain yield declined due to direct competition with the melon vines. Swennen (1990) found mulching most efficient in weed control because a mulch layer impedes or prevent weed growth. Anderson (1996) recommended mulching as an efficient weed control technique and mentioned several other mulched materials that could be used as hay, manure, grass clippings, sawdust, wood chips, straw, rice hulls, paper and plastic films. De La Pena (1978) reported good control of different weed species with the use of glyphosate (Roundup) applied at the rate of 3-5 litres/ha for *Cyperus* sp or at the rate of 5-7 litres/ha for *Cynodon dactylon* (L) in bananas without injury. Baiyeri *et al.* (2004) also conducted a study that produce good results on some yield and phenological traits of *Musa* species under alley and sole cropping management system in Nigeria.

2.10 Major Diseases and Pests of Banana in Africa

Diseases and pests, in combination with poor soil fertility, are responsible for significant reduction in banana yield in Africa (Stover, 2000). Productivity in Central and Southwestern Uganda, for instance, was estimated to be 6 t/ha, which is well

below the potential of 60 t/ha (Viljoen, 2010). This situation, sadly, has improved little since the first research efforts almost 50 years ago (Stover, 2000). Rather, an important new disease, Xanthomonas wilt, which spreads rapidly throughout Central and Eastern Africa was discovered in 2001, causing losses to production and hardship to many small-scale growers in the region (Tushemereirwe *et al.,* 2004). In addition, damag in Banana are caused by diseases such as black leaf streak and Fusarium wilt, and pests such as the banana weevil and plant-parasitic nematodes (Viljoen, 2010). Since the 1970s, banana productivity in sub-Saharan Africa has declined, while population growth increased (Karamura, 1998). The decline in yield/ha could mainly be attributed to low-input application. With a significant decline in soil fertility, and an increase in pathogen and pest damage, more land was planted to banana with little effort made to increase yields in existing fields. The suckers primarily used as planting material were often infected with pathogens and pests (Viljoen, 2010).

2.10.1 Viral diseases affecting banana

All the important banana viral diseases occur in Africa, including Banana bunchy top virus (BBTV), Banana streak virus (BSV), Cucumber mosaic virus (CMV) and Banana die back (BDBD). BSV was first reported in Ivory Coast in 1968 and BBTV in Gabon and Democratic Republic of Congo in 1982 (Manser, 1982) and banana dieback in Nigeria in 1998 (Hughes *et al.,* 1998). BSV and BBTV are both widely distributed on the continent, and affect most *Musa* cultivars (Lockhart and Jones, 2000). Banana viruses are disseminated by insects, infected planting material and on field equipment, and cause significant damage in some areas. BBTV is particularly severe in some Southern African countries, such as Malawi but does not yet occur in others, such as South Africa. CMV occurs sporadically in most countries. Virus

diseases of bananas can be controlled by using clean planting material, eradication of infected plants, control of vectors and good sanitation practices (Thomas *et al.,* 2000).

2.10.2 Banana bunchy top virus (BBTV, Nanoviridae)

Banana bunchy top virus (BBTV; genus Babuvirus, family Nanoviridae), is one of the most serious pathogens of banana (AAA genome) and plantain (AAB genome). It is the most destructive pathogenic virus of banana plants worldwide (Shizu, 2013). BBTV is widespread in Southeast Asia, the Philippines, Taiwan, most of the South Pacific islands, and parts of India and Africa. It is well established in central and southern sub-Saharan Africa (Kumar *et al.,* 2011) in countries like: Angola, Malawi and Zambia in Southern Africa and was reported in Republic of Benin and first reported in Nigeria in 2013 (Adegbola *et al*., 2013). The disease is caused by systemic infection of the plant by virus pathogen (Sadik *et al.,* 2012) and it is transmitted by the banana aphid, *Pentalonia nigronervosa* Coquerel (Homoptera: Aphididae), in a persistent manner (Lokossou 2012). The Virus is not transmitted by mechanical inoculation (ICTVdB, 2006).

2.10.2.1 Symptoms of BBTV

The initial symptoms of banana bunchy top virus consist of dark-green streaks in the veins of lower portions of the leaf midrib and the leaf stem (petiole). The streaks also occur, but are less prominent, in the veins of the leaf blade (lamina). This symptom is sometimes referred to as “Morse code streaking” because the streaks are irregular and resemble a series of “dots” and “dashes.” Rubbing away the waxy white coating that covers the petiole or midrib makes it easier to see the streaking. Also, dark-green, hook-like extensions of the leaf lamina veins can be seen in the narrow, light-green

zone between the midrib and the lamina. The short hooks point down along the midrib toward the petiole. They can best be seen by back-lighting the leaf against the sky. On mature plants infected with BBTV, new leaves emerge with difficulty, are narrower than normal, are wavy rather than flat, and have yellow (chlorotic) leaf margins. They appear to be "bunched" at the top of the plant, the symptom for which this disease is named. Severely infected banana plants usually will not fruit, but if fruit is produced, the banana hands and fingers are likely to be distorted and twisted or it does not completely emerge from the pseudostem (Brooks, 1999). The suckers that develop after a "mother" plant has been infected with BBTV are usually severely stunted, with leaves that do not expand normally and remain bunched at the top of the pseudostem. These leaves are stiff and erect, are shorter and narrower than normal leaves, and have chlorotic edges. Suckers with these symptoms will not produce fruit (Anonymous, 1997).

2.10.3 Banana streak virus (BSV, Caulimoviridae)

Banana streak disease (BSV) was first reported in the Cote d'Ivoire in 1966 and is found in all countries where bananas are grown. Banana streak virus, a plant virus with bacilliform particles and a circular dsDNA genome of 7.4 kbp, is a member of the genus Badnavirus in the family Caulimoviridae. It is the causal agent of banana streak disease which occurs worldwide in banana and plantains (*Musa* spp.) and is widespread in Africa. The only known hosts of BSV are within the family Musaceae. Estimated yield losses of between 7-90 % have been attributed to the disease (Harper *et al.,* 2004). This range probably reflects difference in cultivars and cultural conditions. Most recently, genetic variants of BSV have been reported in Mauritius

(Jaufeerally-Fakim *et al.,* 2006). Banana streak virus (BSV) can either be episomal or integrated to the *Musa* host genome (Agindotan *et al.,* 2006).

BSV can be transmitted experimentally by the mealybugs *Planococcus citri* Risso and *Saccharicoccus sacchari* Cockerell but not by mechanical inoculation of sap extracts. Mealybugs are found on banana and plantain growing in the field in West Africa. In Nigeria the main species are predominantly *Ferrisia virgata* Cockerell*, Dysmicoccus brevipes* Cockerell and *D.grassii* Leonardi. According to Lockhart and Jones (2000), the numbers and proportion of nymphs of these species varies according to the time of year. The main means of transmission of BSV in Nigeria appears to be through dissemination, by farmers, of BSV-infected suckers. Water stress and sustained cooler temperatures (Dahal *et al.,* 1998) seem to be the cause of localized 'outbreaks' of symptoms. Where there remains an adequate supply of water and other stresses are minimised, the plants generally appear to be healthy with few if any symptoms, except during the cooler season in southern Nigeria. Some varieties/hybrids appear to be more susceptible to the stress and cooler temperature induction of symptom expression than others.

The virus is serologically and genomically very variable with many different isolates. Lockhart and Olszewski, (1993) recognised at least 3 distinct serotypes and four distinct isolates based on PCR amplification with degenerate primers followed by a DNA hybridisation analysis.

2.10.3.1 Symptoms of BSV infection in banana

The disease symptom varies and is thought to depend upon a variety of factors such as virus isolate, host genotype, level of management and environmental conditions.

Little is known about the effects of host genotype on symptom expression but expression of symptoms can be intermittent possibly due to environmental conditions such as temperature. This intermittent symptom expression was correlated with virus titre in the leaves with symptomatic leaves containing higher levels of virus.Banana Streak Virus-infected *Musa* plants frequently express broken or continuous chlorotic or necrotic streaks on the leaves, stunting of diseased plants and occasionally heart-rot of the pseudostem and plant death (Lockhart and Jones, 2000), splitting of the outer leaf sheaths of the pseudostem, constriction of the bunch on emergence (choking) and abnormal emergence of the bunch from the middle of the pseudostem, bearing fewer fruits of smaller size (Jones and Lockhart, 1993). The leaf lamina may also be narrower, thicker and become torn and altered phyllotaxis as the leaves arranged in a single vertical plane instead of the normal spiral pattern. In severe instances, cigar leaf necrosis, internal necrosis and collapse of the pseusodstem are observed. Infection can cause a delay in bunch emergence and harvest, a reduction in bunch weight, and shorter and abnormally shaped fruit with a thinner fruit peel that is prone to splitting. In some instances, bunches burst out midway up the pseudostem.

Foliar symptoms are periodic in expression. Periods of symptom expression do follow distinct patterns, but the underlying reasons for these patterns are unclear. In Nigeria, greater symptom expression was observed when plants were grown at 22 ℃ in a growth cabinet than in a screenhouse at 28-35 ℃ (Dahal *et al*., 1998a) and in the cooler wet season compared with the hot dry season (Dahal *et al*., 1998b, 2000). However, contrasting correlations with temperature were observed in Morocco and Australia, where greater symptom expression occurred during hotter times of the year (Lockhart, 1986; Daniells *et al*., 2001). In a study conducted by Daniells *et al*., (2001), only 45-51 % of the leaves produced per season showed symptoms. Daniells

et al. (2001) noted that over two plant generations, symptom expression peaked at the time of bunch initiation, suggesting that plant developmental stage may be a factor in symptom expression.

2.10.4 Cucumber mosaic virus (CMV, Bromoviridae)

Cucumber mosaic virus is a multicomponent virus with a single stranded positive-sense RNA genome consisting of three RNA species plus a forth genomic RNA. Infection with CMV is found in a wide range of crops, including banana. Infectious chlorosis disease of banana was first reported in New South Wales, Australia in 1930, and later in south and central America, the Caribbean, India and the Philippines. In Western Australia, it was first reported in 1988. Infectious chlorosis disease of banana caused by CMV has been confused with zinc deficiency in bananas at Carnarvon (Western Australia) in the past and occurs in most plantations with up to 10% of plants infected (Singh *et al.,* 1995).

CMV causes great losses in vegetables, ornamentals and fruits, and it is destructive due to its rapid spread by more than 60 aphid species in the field. Hsu *et al.* (2000) reported that transmission through planting material is also significant in some crop and weed hosts. Cucumber mosaic virus or infectious chlorosis is one of the important and widely distributed viral diseases of banana. CMV is cosmopolitan, and is found wherever bananas are grown. This virus is a member of the Cucumovirus group and belongs to bromoviridae. CMV is easily transmitted by sap inoculation and by aphids in a non- persistent manner (Kaper *et al.,* 1981). In some virus infections, the whole leaf may become chlorotic due to decreased chlorophyll production and the breakdown of chloroplasts. CMV has alternate host and can be readily, transferred by insect vectors. CMV is transmitted in a non-persistent manner by several aphid

species (Palukaitis and Garcia-Arenal, 2003). Even though CMV infecting other than banana can be transferred to many hosts for the purpose of propagation and purification, it is always difficult to transmit the CMV banana isolate to propagation hosts mechanically (Dheepa and Paranjothi, 2010). CMV is transmitted efficiently by *Aphis gossypii* Glover and poorly by *Aphis craccivora* Koch but not at all by *Pentalonia nigronervosa* Coquerel in Puerto Rico (Kaper *et al.,* 1981).. Shukla and Govinda (2000) made a detailed study to establish virus- vector relationship of aphid of CMV. He showed that a short preacquisition fasting (2 hrs) and acquisition threshold (2 min) given to *Acyrthosiphon pisum* Harris increased the efficiency of the aphids and that a single adult aphid can produce infection, although in low percentage and the percentage infection increased with an increase in the number of aphids.

2.10.4.1 Symptoms of cucumber mosaic virus

Symptoms of CMV include: yellow streaking or mosaic patterns on banana leaves, often with a curling of leaf margins. In cool weather, rotting of heart leaves and the centre of pseudo stem (heart rot or sheath rot) also occurs. Fruit do not normally form on infected plants but when they do, they develop necrosis spots and rings (Dheepa and Paranjothi, 2010).

2.10.5 Banana bract mosaic virus (BBrMV, Potyviridae)

Banana bract mosaic virus (BBrMV) is a filamentous virus on banana (Magnaye and Espino, 1990). It is a member of the genus Potyvirus in the family Potyviridae (Thomas *et al.,* 1997). These viruses are unique in the diversity of inclusion bodies that are formed during the infection cycle. A feature shared by all potyviruses is the induction of characteristic pinwheel or scroll-shaped inclusion bodies in the

cytoplasm of the infected cells. These cylindrical inclusion (CI) bodies are formed by a virus-encoded protein and can be considered as the most important phenotypic criterion for assigning viruses to the poty-virus group (Jose *et al.,* 1992).

Thomas *et al.* (2000) reported that BBrMV occurs naturally on cultivated banana (*Musa* spp.). Symptoms have also been observed on the ornamental banana *M. ornate* growing in close proximity to affected cultivated banana Thomas *et al.* (2000). Although no host is known other than *Musa* spp. Baker *et al*. (2008) reported that studies on the host range of BBrMV have not been comprehensive enough to completely rule out the possible existence of non-*Musa* hosts.

The virus causes typical mosaic symptoms on floral bracts. Symptom expression may vary over time and depends on banana genotypes, varieties, temperature and country (Thomas *et al*., 2000). Yield losses of up to 40% have been reported in 'Saba' and 'Cardaba' (ABB genome) cooking banana cultivars (Roperos and Magnaye, 1991). BBrMV is transmitted experimentally in a non-persistent manner by the cotton or melon aphid, *Aphis gossypii* Glover, the corn aphid, *Rhopalosiphum maidis* Fitch, the banana aphid, *Pentalonia nigronervosa* Coquerel and the cowpea aphid, *Aphis craccivora* Koch. BBrMV can be spread over long distances via the international movement of vegetative propagation material, particularly micropropagated plantlets (vitroplants) (Baker, 2008).

2.11 Techniques for the Detection of Plant Virus Diseases

Many methods have been developed for the detection and identification of plant viruses. A single diagnostic test or assay may provide adequate information on the identity of a virus, but a combination of methods is generally needed for unequivocal

diagnosis. Recent advances in techniques for the detection of proteins and nucleic acids have provided an opportunity to develop methods with these qualities for the diagnosis of plant virus diseases. The type of diagnostic test used ultimately depends on resources, facilities, availability of reagents, level of specificity and sensitivity required expertise and skills available to carry out these assays, type and number of samples to be tested, and the amount of information available on the virus to be detected (Naidu and Hughes, 2001).

Technique available for the detection of plant virus diseases used by scientists in developing countries especially in sub-Saharan Africa includes: Symptomatology, Transmission tests, Physical properties, Microscopy, Immunosorbent electron Microscopy, Serology, Rolling circle amplification and Polymerase chain reaction (PCR) technique. Although, widely used for virus detection, serological methods have certain disadvantages. They are based on the antigenic properties of the virus coat protein, which represents only about 10% of the total virus genome (Gould and Symons 1983). The sensitivity of nucleic acid-based detection systems was greatly improved following the development of the polymerase chain reaction (PCR) procedure (Mullis *et al.,* 1986).

2.11.1 Polymerase chain reaction (PCR) for the detection of plant virus diseases

PCR is an *invitro* method for amplifying target nucleic acid sequences. The speed, specificity, sensitivity, and versatility of PCR made it suitable in many areas of research in biology. PCR has the power to amplify the target nucleic acid present at an extremely low level (Candresse *et al*., 1998a). PCR consists of three steps: Denaturation, annealing and extension of each primer. The three step cycles are repeated many times (between 30 and 40 cycles) in an automated thermal cycler until

sufficient product is produced. Thus a single template molecule will be amplified 2^n times after n cycles, i.e., approximately 3.4 ×1010 times in 35 cycles if it is assumed that the reaction proceeds with 100% efficiency. However, the efficiency typically spans the range of 65–85% and one can expect that the total amount of product synthesized would be between 1.65^n and 1.85^n (Krawetz 1989). This procedure is applicable directly to DNA plant viruses (caulimo, gemini, and badnaviruses); however, for diagnosis of plant viruses with RNA genomes, the RNA target has to be "converted" to a complementary DNA (cDNA) copy by reverse-transcription before PCR is begun. The cDNA provides a suitable DNA target for subsequent amplification. During the initial cycles of PCR, a complementary strand of DNA will be synthesized from the cDNA template, and thereafter the reaction will proceed as for double-stranded DNA described above. This process of amplification is called reverse transcription-polymerase chain reaction (RT-PCR). On completion of the reaction, the amplified DNA can be analyzed by agarose gel electrophoresis.

2.12 Managing Banana Diseases in Africa

To reduce losses in banana yield and quality due to diseases, the pathogen or pest involved has to be considered along with the banana production system. For instance, commercial farmers can afford disease-free tissue culture plants and pesticides for control of nematodes and black leaf streak. However, such methods of disease control are seldom an option for resource-poor farmers. For these producers, disease-resistant cultivars and cultural management provide the most efficient ways of reducing losses (Viljoen, 2010).

2.12.1 Disease-resistant cultivars

As a commodity with a clonal means of reproduction, bananas have a low level of genetic diversity to exploit for disease and pest control. Within preferred banana types (cooking, dessert and beer bananas, and plantains), most cultivars will either be immune or susceptible. For instance, EAHB and plantains show immunity to Fusarium oxysporum f.sp. cubense (Tushemereirwe and Bagabe, 1998), while dessert bananas are all susceptible (Ploetz and Pegg, 2000). The response of Banana to black leaf streak and nematodes can vary from highly susceptible to resistant (Carlier *et al.,* 2000; Sarah, 2000). Disease-resistant cultivars can be developed through conventional and unconventional breeding methods, depending on whether sources of resistance are available in banana. While good resistance against Fusarium wilt and black leaf streak has been introduced at banana breeding programs, disease-resistant hybrids take a long time to develop and are often not popular at local markets. Unconventional improvement through mutation breeding and genetic modification can reduce the time and costs of conventional breeding substantially (Crouch *et al.,* 1998), but stability of traits against pathogens and pests, and acceptance of such plants by the community is not guaranteed.

2.12.2 Chemical control

Banana diseases such as black leaf streak require intensive fungicide spray programs to enable commercial productivity (Carlier *et al.,* 2000). Without this, yields of plants affected can be reduced by as much as 100% of the potential yield. For some diseases, such as Fusarium wilt and Xanthomonas wilt, pesticides are ineffective. Even if effective, chemical control is available to poor farmers in rural areas it is often not feasible. Pesticide use is also becoming of increasing concern with regard to the

environment and human health, with alternative disease and pest control options becoming increasingly sought. Over-exploitation of pesticides have also led to resistance build-up in pathogens such as in *Mycosphaerella fijiensis* (Carlier *et al.,* 2000), and in pests such as *Cosmopolites sordidus*, thereby reducing their effectiveness.

2.12.3 Biological control

With the general disapproval of pesticide used, biological control of pests and diseases has grown in popularity. The banana weevil can be controlled using either predators (Hasyim and Gold, 1999) or entomopathogenic fungi, such as *Beauveria bassiana* (Nankinga and Moore, 2000). However, an area where significant progress has been made is in the use of fungal endophytes, in particular non-pathogenic *Fusarium oxysporum*, against nematodes, *C. sordidus* and Fusarium oxysporum f.sp. cubense (Foc) in banana (Paparu *et al.,* 2009). The principle of plant bio-enhancement using non-pathogenic *F. oxysporum* is that the endophyte can be established inside the roots of micro-propagated banana plants en mass, and that the endophyte will establish itself in planta to protect it against potential root and rhizome invaders for a significant period of time. This approach, thus, negates the need for continuous application. Biological control has not yet achieved its full potential in disease and pest control, and requires significantly greater research efforts to become a realistic replacement for pesticide use in commercial banana production (Paparu *et al.,* 2009).

2.12.4 Cultural control

Cultural control options offer a primary, affordable and most practical means for disease and pest management in Africa. Planting material, in particular, is treated by

cutting away affected roots and tissue from banana suckers before treating with hot water (Gold *et al.,* 2003) or boiling water (Hauser, 2007). Unfortunately, symptomless plants infected with viruses or fungal pathogens such as BSV and Fusarium oxysporum f.sp. cubense (Foc), can unwittingly be introduced into new areas. It is, thus, of great importance that awareness campaigns are put into action as was successfully achieved for the control of Xanthomonas wilt in Uganda (Tripathi *et al.,* 2009). Such awareness campaigns require sustained follow-up actions to ensure that renewed outbreaks do not occur. Xanthomonas wilt was brought under control through a concerted program involving sustained input from local, national and regional actors to provide crossborder coverage in a region hit heavily by the disease. A combination of management options also helped bring the outbreak under control, such as the early removal of male buds, proper sanitation practices and destruction of affected material (Biruma *et al.,* 2007). Leaf pruning helps reduce damage caused by leaf diseases, as can reduce planting density and proper field drainage (Carlier *et al.,* 2000). When present in fields, nematodes can be managed through use of mulches and botanicals, intercropping and crop rotation, and leaving land fallow. Trapping banana weevils is amongst the most effective cultural control methods (Gold *et al.,* 2003).

2.12.5 Disease-free planting material through meristem culture

One of the most important means of disease and pest control is to prevent the introduction of pests and diseases in newly established banana fields by using healthy, uninfected planting material. Tissue culture plants are considered pathogen- and pest-free, as they are produced under sterile laboratory conditions and often indexed to ensure that they are virus-free. Once planted, tissue culture banana plants are,

however, vulnerable to attack by pathogens and pests that are already present or introduced to the newly planted banana field (Viljoen, 2010).

CHAPTER THREE

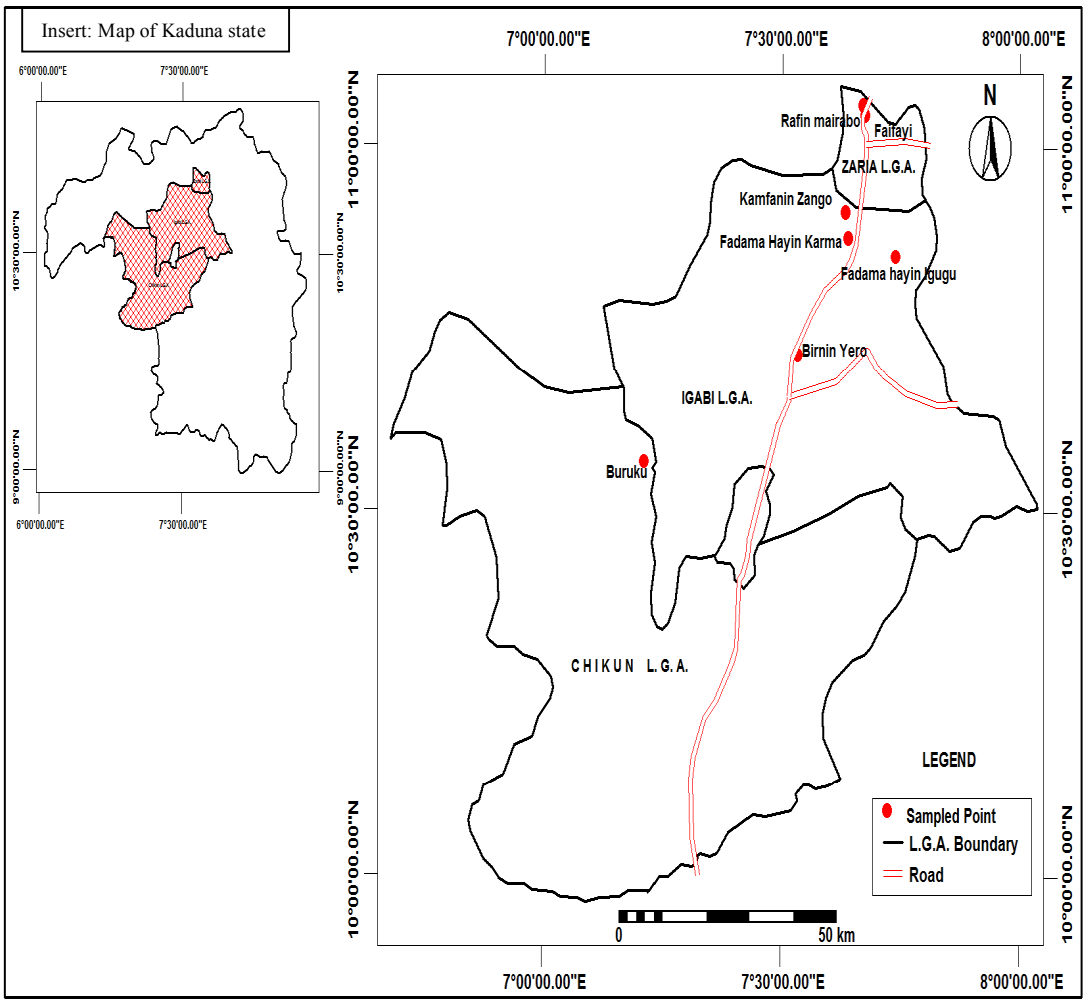
3.0 MATERIALS AND METHODS

3.1 Survey for Banana Viruses

Survey for banana viruses was conducted in three local government areas of Kaduna State between February and July 2013 to determine the incidence of banana viruses (Figure 1). Seven farms belonging to peasant farmers were surveyed. Two farms in Zaria Local Government Area: Rafin Mairabo Farm which lies at 11°05.748' N, 007° 41.338' E and Faifayi (11°02.439' N, 007° 40.926' E),

Four farms in Igabi local government: Kamfanin Zongo Farm (11°54.549' N, 007°38.202' E), Fadaman Hayin Karma Farm (10°88.036' N, 007° 63.881' E), Fadaman Hayin Igugu Farm (10°88.070' N, 007° 63.672' E) and Fadaman Tsallaken Titi Farm (10°77.244' N, 007°52.057'E). A farm in Chukun local government: Buruku Farm (10°61.704' N, 007° 22.681' E)

Structured questionnaire (Appendix 1) was administered to farmers to ascertain the farming system, pests and diseases associated with banana cultivation. Leaf samples were collected after count of 5 or 15 stands (Lokossou *et al*., 2012) depending on size of the farm. Both symptomatic and asymptomatic banana leaves were collected from the field. Weeds around banana stands were also collected. Insect found associated with banana plants were collected and preserved in 70% alcohol. Samples were placed in polythene bags after collection, labelled and preserved both frozen and under Calcium chloride. Incidence of each disease was recorded based on symptom expression within each field using the formula:



Source: Adapted and modified from Administrative Map of Kaduna State

Fig 1: Sample sites in the 3 L.G.As of Kaduna state, Nigeria

Percentage disease incidence= Number of diseased units x 100

Total assed units

For the symptomatic samples, the types of symptoms observed on all the symptomatic plants were recorded. To quantify symptom severity, each sample was scored on a scale of 0-5 (Karanja, 2010), where 0 is no symptoms, 1 is localised flecks, 2 is scattered discontinuous streaks, 3 is continuous streaks covering moderate portion of lamina, 4 is continuous chlorotic streaks, and 5 is necrotic streaks (Appendix 2).

3.2 Detection of Banana Viruses from Banana Samples using Polymerase Chain Reaction (PCR)

The methods used were PCR for BBTV and BSV while RT-PCR for CMV and Potyvirus detection of both *Musa* and weed samples. The procedures for PCR were according to Hughes *et al.* (2004).

3.2.1 Total nucleic acid extraction

Cetyl trimethyl ammonium bromide (CTAB) buffer pH 8.0 was used for the extraction of total nucleic acid according to Abarshi *et al.* (2010). One hundred milligrams of leaf tissue was grinded in 1000 µl of extraction buffer in a sterile mortar and pestle. The sap was poured into new sterile eppendorf tube and votexed briefly. The extract was incubated in water bath at 60 ^{o}C for 10 minutes. This was brought to room temperature and equal volume of phenol, chloroform and Iso-amyl alcohol (25:24:1) mixture was added. This was votexed and centrifuged at 12000 g for 10 mins. the supernatant (450 µl) was pipetted into a new sterile eppendorf tube. 300 µl of cold isopropanol was added into the tube. This was mixed gently and incubated for 1 hr at -20 ^{o}C. It was centrifuged at 12000 g for 10minutes to sediment the DNA. The supernatant was decanted gently to ensure that the pellets were not disturbed. The

pellets were washed by adding 500 µl of 70 % ethanol and centrifuged at 12000 g for 5 minutes (this was to wash it). Ethanol was decanted and the DNA precipitate was air dried at room temperature. The pellets were re-suspended in 50 µl sterile distilled water for further use and storage

3.2.2 Determination of quality and quantity of DNA extracted

3.2.2.1 Integrity test

Integrity test was used to determine the quality of DNA extracted. A bottle containing 100 ml of TAE buffer and 1 g of agarose powder was put in microwave and set for 5 min. Ethidium bromide (3 µl per 100 ml) was added to the mixture for staining and visualisation of the gel. The ethidium bromide and TAE buffer were mixed thoroughly. Gel comb was inserted into gel plate and the mixture added to the plate. This was allowed to solidify for 20 min. The comb was removed from the gel. The gel was inserted into electrophoretic tank. 4 µl of DNA was mixed with 4 µl of loading dye on a parafilm using micropipette. 8 µl of the mixture for each sample was pipetted into separate well of the gel. The electrophoretic machine (Bio Rad, Model 200/2.0) was set at 110 volts and ran for 30 min. The gel was photographed using Gel documentation machine (Gel Doc EZ imager Bio- Rad) for gel analysis to determine the quality of DNA

3.2.3.2 Nano drop

The quantity of DNA or RNA in each sample was determined using Thermo Scientic Nano drop 2000. 1 µl of sterile distil (as blank) was loaded into nano drop tip. After blanking, 1 µl of sample was added into the nano drop tip. The programme was Ran and the detail for the sample was recorded.

3.3 Detection of Banana Bract Mosaic Virus (BBrMV) by Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)

3.3.1 Total nucleic acid extraction

The extracted RNA from the samples in 3.2.1 above was subjected to PCR analysis.

3.3.2 Primers used for BBrMV detection

For RT-PCR amplification of potyviruses, RT-PCR was used (Alexandre *et al*., 2013). The primers used are the cylindrical inclusion (CI) for the detection of potyviruses.

CI F: (CGIVIGTIGGIWSIGGAARTCIAC)

CI R: (ACICCRTTYTCDATDATRTTIGTIGC)

3.3.3 RT-PCR reaction mixture for banana samples

Sterile distilled water	6.38 µl
Buffer	2.5 µl
dNTPs	0.25 µl
$MgCl_2$	0.75 µl
CI F	0.25 µl
CI R	0.25 µl
Taq	0.06 µl
RT	0.06 µl

Reverse transcriptase enzyme was added to the reaction mixture in order to reverse transcribe the single stranded RNA of BBrMV into double stranded cDNA.

Suspended extracted RNA was votexed. 2 µl of each sample was pipetted in 0.6ml eppendorf tube. 98 µl of sterile water was added into each sample (1:50 dilution) in a serial dilution. Reaction mixture consisting of primers, buffer, dNTPs, $MgCl_2$, and water was prepared, votexed and spin down. 10.5 µl of reaction mix was added into

each PCR tube and 2 µl of each sample was added into the PCR tubes containing the reaction mixture. The PCR tubes were covered, spinned and amplified in PCR machine (Applied Biosystem Veriti 96 well thermal cycler)

3.3.4 PCR cycles for BBrMV

The RNA of the virus was Reverse Transcribed at 42º C for 30 mins followed by denaturation of DNA at 94 ºC for 3 min. followed by 40 cycles annealing at 94 ºC for 30 secs, 40 ºC for 30 secs, 68 ºC for 1 min and finally an extension at 72 ºC for 10 mins. The amplified DNA was stored at 4 ºC for later use.

3.3.5 Analysis of RT-PCR product

The amplified RNA were electrophoresed on 1.5% agarose gel with 1x TAE buffer, stained with ethidium bromide and photographed using gel documentation machine (Gel Doc EZ imager Bio- Rad). The molecular weight of the PCR product was determined by comparison with DNA markers 100 bp ladder.

3.4 Detection of Cucumber Mosaic Virus (CMV) by Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)

3.4.1 RT-PCR for CMV

The extracted RNA from the samples in section 3.2.1 above was subjected to PCR analysis.

3.4.2 Primers used for CMV detection

Oligonucleotide primers specific for CMV were used for RT-PCR amplification of CMV coat protein gene (Wylie *et al.,* 1993).

CMV 1: (5′ GCCGTAAGCTGGATGGACAA 3′).

CMV 2: (5′ TATGATAAGAAGCTTGTTTCGCG 3′)

3.4.3 RT-PCR reaction mixture for CMV

The reaction mixture and the RT-PCR procedure were carried out as described in section 3.3.3 above.

3.4.4 PCR cycles for CMV

The RNA of the virus was Reverse Transcribed at 50 ºC for 10 mins followed by denaturation of DNA at 95 ºC for 5 min. followed by 35 cycles annealing at 95 ºC for 30 secs, 55 ºC for 1 min 72 ºC for 1 min and finally an extension at 72 ºC for 10 mins. The amplified DNA is stored at 4 ºC for later use.

3.4.5 Analysis of RT-PCR product

Analysis of RT-PCR product was carried out according to the procedure described in section 3.3.5 above.

3.5 Detection of BBTV by PCR

3.5.1 Total nucleic acid extraction

The extracted DNA from the samples in section 3.2.1 above was subjected to PCR analysis.

3.5.2 Primers used for BBTV detection

Scp-F and Scp-R specific for a 1075 bp BBTV DNA that encodes coat protein gene (Amin *et al.,* 2008).

Scp F: CCGGGAGAATACTTCACTGGGCTATAGAT,

Scp R: CCCGGCTTCACCTTGCACACCAACAGCAT

3.5.3 PCR reaction mixture for BBTV

The reaction mixture and the RT-PCR procedure were carried out as described in section 3.3.3 above. However for BBTV, reverse transcriptase enzyme was not added to the reaction mixture as BBTV is a DNA virus. The concentrations of reaction

mixture constituents are same as in 3.3.3 above with the exception of sterile distilled water which has a volume of 6.44 µl

3.5.4 PCR cycles for BBTV

Temperature was increased to 95 ºC for 5 mins for denaturation of DNA followed by 35 cycles of 95 ºC for 1 min, 52 ºC for 1 min and 72 ºC for 1 mins for annealing. Afterwards an extension at 72 ºC for 10 mins. The amplified DNA is stored at 4 ºC for later use.

3.5.5 Analysis of PCR product

Analysis of RT-PCR product was carried out according to the procedure described in section 3.3.5 above.

3.6 Detection of Banana Streak Virus (BSV) by Polymerase Chain Reaction (PCR)

3.6.1 Total nucleic acid extraction

The extracted DNA from the samples in 3.2.1 above was subjected to PCR analysis.

3.6.2 Primers used for BSV detection

BSV-4673 known as (BSV F): 5'-GGAATGAAAGAGCAGGCC-3' and

BSV-5317 known as (BSV R): 5'-AGTCATTGGGTCAACCTCTGTCCC-3' are the sequence detail for the primer used for PCR amplification of BSV (BSV Onne primers; Harper *et al.,* 1998),

3.6.3 PCR reaction mixture for BSV

The reaction mixture and the RT-PCR procedure were carried out as described in section 3.3.3 above. However for BBTV, reverse transcriptase enzyme was not added to the reaction mixture as BBTV is a DNA virus. The concentrations of reaction

mixture constituents are same as in 3.3.3 above with the exception of sterile distilled water which has a volume of 6.44 µl

3.6.4 PCR cycles for BSV

Temperature was increased to 94 ºC for 4 mins for denaturation of DNA followed by 35 cycles of 94 ºC for 1 min, 50 ºC for 1 min and 72 ºC for 2 mins for annealing. Afterwards an extension at 72 ºC for 5 min and finally the amplified DNA was stored at 4 ºC for later use.

3.6.5 Analysis of PCR product

Analysis of RT-PCR product was carried out according to the procedure described in section 3.3.5 above.

3.7 Serological Techniques: Antigen Coated Plate Enzyme Linked Immunosorbent Assay (ACP ELISA)

ACP ELISA was carried out on weed samples positive for Potyvirus and CMV through PCR according to the procedure of Hughes, (2004). 100 µl of antigen was grounded in coating buffer (or coating buffer +2% PVP) and put into ELISA plate. Plate containg the antigen was covered and incubated overnight at 4 ºC. The plate was washed three times with PBS-Tween by flooding for 3 min each time. Plate was emptied and tap dry. Each well was blocked with 200 µl of 3 % dried skimmed milk in PBS-Tween. Plate was covered and incubated at 37 ºC for 30 min and tapped dry. 100 µl of polyclonal antibody diluted 1: 50 in conjugate buffer was added per well. Plate was covered and incubated for 2hrs at 37ºC. Plate was washed 3 times with PBS-Tween by flooding for 3 min each time the plate is tapped dry. 100 µl per well of goat anti rabbit alkaline phosphate conjugate diluted in conjugate buffer was added. Plate was covered and incubated at 37 ºC for 2 hr. 200 µl of 0.5-1 mg/mol of p-

nitrophenyl phosphate substrate in substrate buffer was added per well. The plate was read after one hour and overnight.

3.8 Detection of Banana Streak Viruses from Mealybugs by Polymerase Chain Reaction (PCR)

3.8.1 Total nucleic acid extraction

Mealybug DNA was extracted using the method by Dellaporta *et al*. (1983). 20 µl of DNA extraction buffer was added to mealybug and macerated manually for 3-5 minutes. 300 µl of extraction buffer was used to wash the pestle and sides of the tubes after maceration. 33 µl of 20 % sodium dodecyl sulphate was added, votexed and incubated at 65^{o}C for 10minutes in water bath. This was brought to room temperature and 160 µl of 5M potassium acetate was added, votexed and spinned at 10000 g for 10 min. Afterwards, 200 µl of supernatant was pipetted into a new tube. 100 µl of cold isopropyl alcohol was added. This was mixed gently and incubated at 4^{o}C for 20 min. The extract was spinned at 10000 g for 10 min. The cold iso propanol was carefully decanted leaving the DNA pellet. 500 µl of 70% alcohol was added for washing the pellet and spinned for 5 minutes at 10000 g. The 70% alcohol was decanted and pellet dried in incubator at 37^{o}C for 15 minutes. Pellets were resuspended in sterile distilled water for later use.

3.8.2 Determination of quality and quantity of DNA extracted

3.8.2.1 Integrity test

Integrity test was carried out according to the procedure described in section 3.2.2.1

3.8.2.2 Nano drop test

The quantity of DNA in each sample was determined using Thermo Scientic Nano drop 2000. The procedure was carried out according to the procedure described in section 3.2.2.1

3.8.3 Detection of BSV by PCR

The extracted DNA in 3.8.1 was subjected to PCR analysis.

3.8.4 Primers used for BSV detection

BSV-4673 known as (BSV F): 5'-GGAATGAAAGAGCAGGCC-3' and

BSV-5317 known as (BSV R): 5'-AGTCATTGGGTCAACCTCTGTC-3' are the sequence detail for the primer used for PCR amplification of BSV (BSV Onne primers; Harper *et al.,* 1998),

3.8.5 PCR reaction mixture for BSV for Mealybug samples

The reaction mixture and the RT-PCR procedure were carried out as described in section 3.3.3 above. However for BBTV, reverse transcriptase enzyme was not added to the reaction mixture as BBTV is a DNA virus. The concentrations of reaction mixture constituents are same as in 3.3.3 above with the exception of sterile distilled water which has a volume of 6.44 µl

3.8.6 PCR cycles for BSV

Temperature was increased to 94 ºC for 4 min for denaturation of DNA followed by 35 cycles of 94 ºC for 1 min, 50 ºC for 1 min and 72 ºC for 2 min for annealing. Afterwards an extension at 72 ºC for 5 min and finally the amplified DNA were stored at 4 ºC for later use.

3.8.7 Analysis of PCR product

Analysis of RT-PCR product was carried out according to the procedure described in section 3.3.5 above.

3.9 Detection of Banana Bunchy Top Virus from Aphids by Polymerase Chain Reaction (PCR)

3.9.1 Total nucleic acid extraction

Total nucleic acid was extracted according to the procedure described in section 3.8.1 above.

3.9.2 Determination of quality and quantity of DNA extracted

3.9.2.1 Integrity test

Integrity test was carried out according to the procedure described in section 3.2.2.1

3.9.2.2 Nano drop test

The quantity of DNA in each sample was determined using Thermo Scientic Nano drop 2000. The procedure was carried out according to the procedure described in section 3.2.2.1

3.9.3 Detection of BBTV by PCR analysis

The extracted DNA in 3.9.1 was subjected to PCR analysis.

3.9.4 Primers used for BBTV

Scp-F and Scp-R specific for a 1075 bp BBTV DNA that encodes coat protein gene (Amin *et al.,* 2008).

Scp F: CCGGGAGAATACTTCACTGGGCTATAGAT,

Scp R: CCCGGCTTCACCTTGCACACCAACAGCAT

3.9.5 PCR reaction mixture for BBTV for samples

The reaction mixture and the RT-PCR procedure were carried out as described in section 3.3.3 above. However for BBTV, reverse transcriptase enzyme was not added

to the reaction mixture as BBTV is a DNA virus. The concentrations of reaction mixture constituents are same as in 3.3.3 above with the exception of sterile distilled water which has a volume of 6.44 µl

3.9.6 PCR cycles for BBTV

Temperature was increased to 95 ºC for 5 min for denaturation of DNA followed by 35 cycles of 95 ºC for 1 min, 52 ºC for 1 min and 72 ºC for 1 min for annealing. Afterwards an extension at 72 ºC for 10 min. The amplified DNA is stored at 4 ºC for later use.

3.9.7 Analysis of PCR product

Analysis of RT-PCR product was carried out according to the procedure described in section 3.3.5 above.

3.11 Data Analysis

Analysis of variance (ANOVA) was carried out to determine the significant difference between samples collected from different locations using SPSS version 20 (2011).When significant, the Duncan's multiple range test (DMRT) was used to separate the means.

CHAPTER FOUR

4.0 RESULTS

4.1 Survey for Pests and Diseases of Banana in the Study Areas

4.1.1 Farming system adopted by banana farmers in the three LGA surveyed

Farming system adopted by farmers has been summarized in Table 4.1. A total of seven farms were surveyed. All the farms visited were owned by men and not women. Most of them reported to have inherited the farm from their parents and have been in possession of the farm for over 15 years. All the farmers practiced mixed cropping. Bananas were intercropped with tomato, pepper and sugarcane farms and now they are grown as hedge around sugarcane fields (Plate I).

4.1.2 Types of banana grown in the areas

Two local land races of banana were grown by banana farmers: the dessert banana (Plate IIa) and tall banana with short fingers called Iyar Lagos (Plate IIb). 44% of the farms surveyed grow only the shorter land race while 66% grow both local land races (short and tall banana). Local land race (dessert banana) were grew in Faifayi, Kamfanin Zongo of Zaria Local Government and Buruku of Chikun Local Government areas while both dessert and tall banana were grown at Rafin Mairabo, Hayin Karma, Hayin Igugu and Tsallaken Titi of Igabi Local Government area. Out of the seven farms surveyed, Buruku was the only farm that plantain was grown. All the farmers planted banana for commercial purpose. Bananas were observed to be grown in Fadama areas.

Table 4.1: Farming System Adopted by Banana Farmers in Igabi, Chukun and Zaria L.G.As of Kaduna State.

Farms / Farming system	Rafin mairabo	Rafin Faifayi	Kamfanin zongo	Fadaman Hayin karma	Fadaman Hayin Igugu	Fadaman Tsallaken titi (Birninyero)	Buruku
Name of the farmer	Mal Basiru	Mal Hussaini	Mal Abdurrazak	Mal. Rabiu	Mal Rabiu	Mal Badamasi	Mal Nasiru
Sex	M	M	M	M	M	M	M
Type of *Musa* sp. Grown	Banana	Banana	Banana	Banana	Banana	Banana	Banana and plantain
Years of cultivation	_	Over 15 years	Over 15years	Over 30 years	Over 30years	_	_
Purpose of cultivation	Commercial	Commercial	Commercial	Commercial	Commercial	Commercial	Commercial
Banana Varieties	Local land race and tall banana	Local land race	Local land race	Local land race and tall banana	Local land race and tall banana	Local land race and tall banana	Local land race
Cropping system	Mixed cropping	Mixed cropping	Mixed cropping	Mixed cropping	Mixed cropping	Mixed cropping	Mixed cropping



Plate I: Banana plants used as hedge around sugarcane farm, a- banana plants, b- sugarcane



(a)



(b)

Plate II: Types of Banana cultivated in the studied areas

(a) Local land race (dessert banana)
(b) Tall banana ("Iyar Lagos")

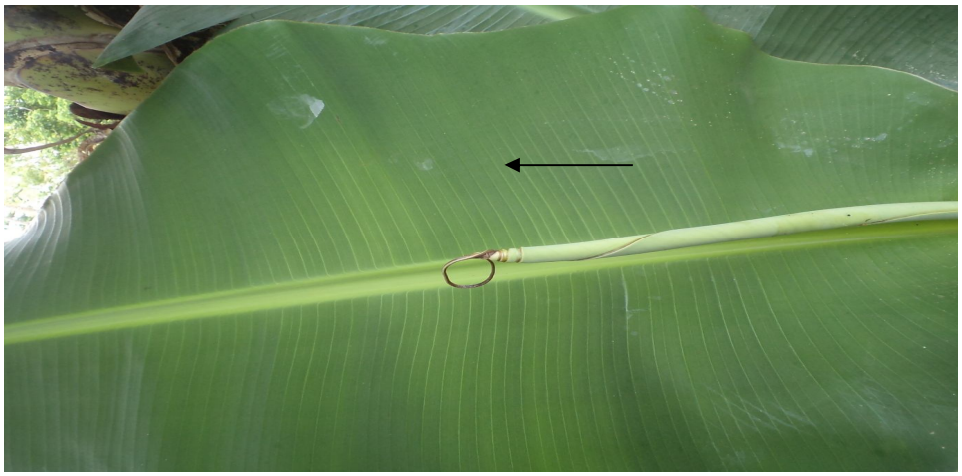
4.2 Problems Encountered by Banana Farmers in the Areas Surveyed

4.2.1 Diseases

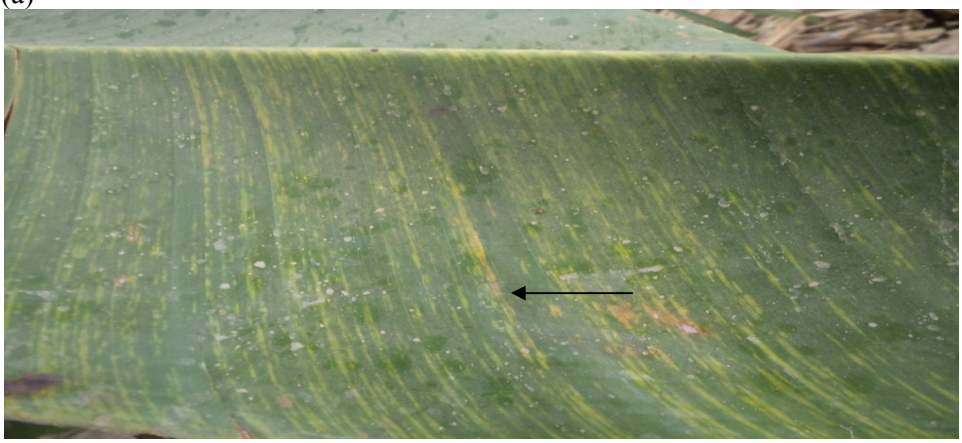
One of the major problems affecting Banana cultivation as reported by banana farmers as well as observation made in the field was disease (Table 4.2). All of the farms visited showed symptoms of disease on leaves, pseudo stem and or peduncle. Typical symptoms of BSV were observed as shown in Plate III in comparison with healthy leaf (Plate IIIa). Chlorotic stripes on banana leaves are an example of BSV (Plate IIIb) and at advanced stage in form of necrotic stripes (Plate IIIc). Mosaic symptom on banana leaves typical of CMV was observed in Kamfanin Zango Farm (Plate IV). Mosaic like symptoms were also observed on some weeds growing around banana stands (Plate V). Symptoms like bulged pseudostem containing peduncle with the fingers (Plate VIa), emergence of bunch from the middle of the pseudostem (Plate VIb) and peduncle twisting (Plate VIc) which are symptoms of BSV were noticed. Symptoms associated with CMV and BSV like: Heart rot (Plate VII) was also observed. Aphids suspected to be vectors of CMV, BBTV and BBrMV were observed on banana pseudostem (Plate VIIIa) as well as on some weeds growing around banana stand (Plates VIIIb and VIIIc). Mealybugs which are the vectors of BSV were observed on banana leaves (Plate IXa) as well as weeds like *Cyperus* sp (Plates IXb) also in the soil (Plate IXc). Other symptoms observed were: Reduced bunch (Plate X) and dry rot of fruit (Plate XI). Having reported the above as problems affecting banana cultivation, however, the farmers were not aware that it is associated with viral symptoms rather, they associated the symptom with water stress, overcrowding as well as insufficient fertiliser

Table 4.2: Problems Encountered by Banana Farmers in the Studied Areas

Farms / Problems	Rafin Mairabo	Faifayi Farm	Kamfanin zongo	Fadaman Hayin karma	Fadaman hayin igugu	Fadaman tsallaken titi	Buruku farm
Source of planting material	Kufena college and old stock	Farm at Abuja and old stock (name not disclosed)	Old stock	Old stock	Old stock	Old stock	Old stock
Source of water	Stream	Stream	Stream	Stream	Stream	Stream	River
Parts of plant infected	Pseudostem, fruit, leaves	Pseudostem, fruit, leaves	Leaves	Pseudostem leaves	Pseudostem, fruit, leaves	Pseudostem , leaves	Leaves
Diseased symptoms observed on crop	Fruit remains in the pseudo stem, heart rot, reduced bunch, Peduncle twisting, fruit dry rot.	Heart rot and small bunch, fruit dry rot	Reduced bunch	Peduncle twisting and small bunch	Fruit dry rot, small bunch and heart rot	No visible sign or symptom	No visible sign or symptom
Causes of the symptoms from farmers perspective	Water stress	Water stress, overcrowding and insufficient fertiliser.	Overcrowding	Water stress	Water stress	Water stress	_
Type of fertiliser used	Organic	Inorganic and organic	Organic	Organic	Organic	Organic	Organic



(a)



(b)



Plate III: Healthy and BSV symptoms on banana leaf

(a) Healthy leaf
(b) Chlorotic stripes on banana leaf due to BSV infection
(c) Necrotic stripes on banana leaf (Advanced stage of BSV symptom)



(a)



(b)

Plate IV: Healthy and CMV banana infected leaf

(a) Healthy banana leaf

(b) Mosaic pattern on banana leaf due to CMV infection



Plate V: Healthy and CMV infected *Centella asiatica* (L)

(a): Healthy leaf
(b): Dark green symptom of CMV



(a)



(b)





(c)

Plate VI: Effect of BSV on pseudostem
(a): Bulged pseudostem containing peduncle with fingers (dessert banana)
(b): Emergence of bunch from the middle of the pseudostem (dessert banana)
(c): Healthy peduncle (1), Peduncle twisting (land race banana) due to BSV infection (2)



Plate VII: Heart rot in short land race banana due to symptom of CMV and BSV



(a)

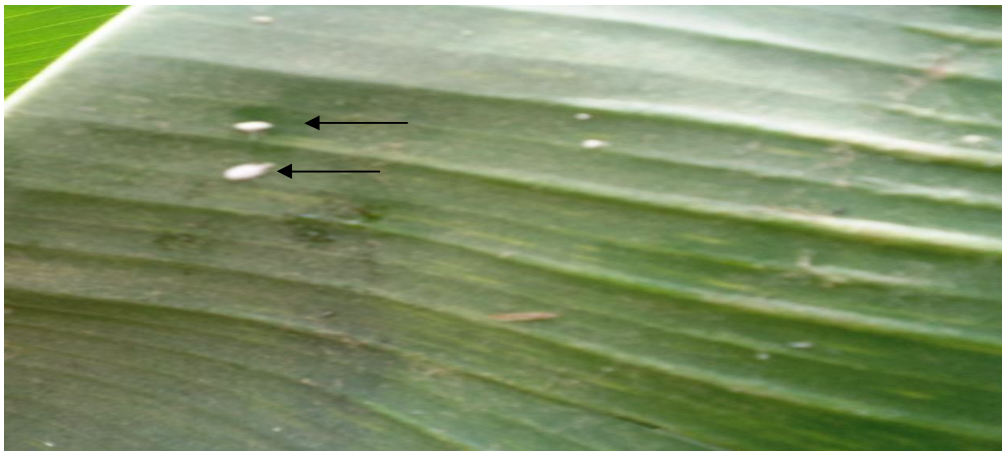


(b)



(c)

Plate VIII: Aphids associated with banana and *Commelina diffusa*
(a): Black aphid (*Pentalonia* spp.) on banana pseudostem
(b): Black aphid (*Pentalonia* spp.) on *Commelina diffusa*
(c): Green aphids (*Myzus persicae*) on the adaxial surface of *Commelina diffusa*



(a)



(b)



(c)

Plate IX: Mealybugs on banana leaf, *Cyperus* sp and soil

(a) Mealy bug (*Planococcus* spp.) on banana leaf

(b) Mealy bug (*Planococcus* spp.) on *Cyperus* sp

(c) Mealy bug (Dysmococcus spp.) on roots of weed found growing near banana plant



Plate X: Reduced bunch (short land race banana)



Plate XI: Fruit dry rot of banana

4.3 Incidence of Banana Viral Diseases in the Studied Areas

Incidence of banana viruses in the areas visited was calculated based on symptoms of BSV, BBTV, CMV and BBrMV observed in the study areas. The result from Table 4.3 showed the highest incidence for BSV in Buruku (Chukun L.G.A) with 88% incidence. CMV follows BSV with Kamfanin Zango (Igabi L.G.A) having 28%. However, there was no symptom of BBTV and BBrMV observed. Birnin yero had no incidence of the four viruses. Faifayi has incidence of only CMV, Zaria LGA had the lowest incidence of both CMV and BSV followed by Igabi LGA while Chukun L.G.A. had the higest incidence for both CMV and BSV. Based on the summary of incidence in the surveyed local government aresas as shown by the mean, BSV was the most important virus (29%) followed by CMV (16%). BSV and CMV were the most widely distributed and also most important in terms of incidence in the areas surveyed.

4.4 Determination of DNA/RNA Quality (Integrity Test)

The quality of the nucleic acid extracted was tested by electrophoresis on 1% agarose gels. Integrity test was conducted on the samples to determine the quality of DNA and RNA that will be subjected to PCR/RT-PCR. Figure 2 and 3 show the result of integrity test for samples. Nucleic acid extraction process were repeated fpr samples that did not show band for DNA, RNA or both which indicates that the nucleic acid is degraded. Sample 3, 5, 9 and 19 in Figure 2 and samples 4, 7, 9, 15, 22,23,25, 26, 27 and 29 in Figure 3 were re-extracted due to the absence of DNA or both DNA and RNA which were lost during the extraction process.

Table 4.3: Incidence of Banana Viral Diseases in the Studied Areas

Local government	Farms	CMV incidence %	BBTV incidence %	BSV incidence %	BBrMV incidence %
Zaria	Rafin mairabo	9	0	18	0
	Faifayi	10	0	0	0
Igabi	Kamfanin zongo	28	0	28	0
	Fadaman hayin karma	18	0	39	0
	Fadaman Hayin Igugu	21	0	36	0
	Birnin Yero	0	0	0	0
Chukun	Buruku	13	0	88	0
Mean		16	0	29	0

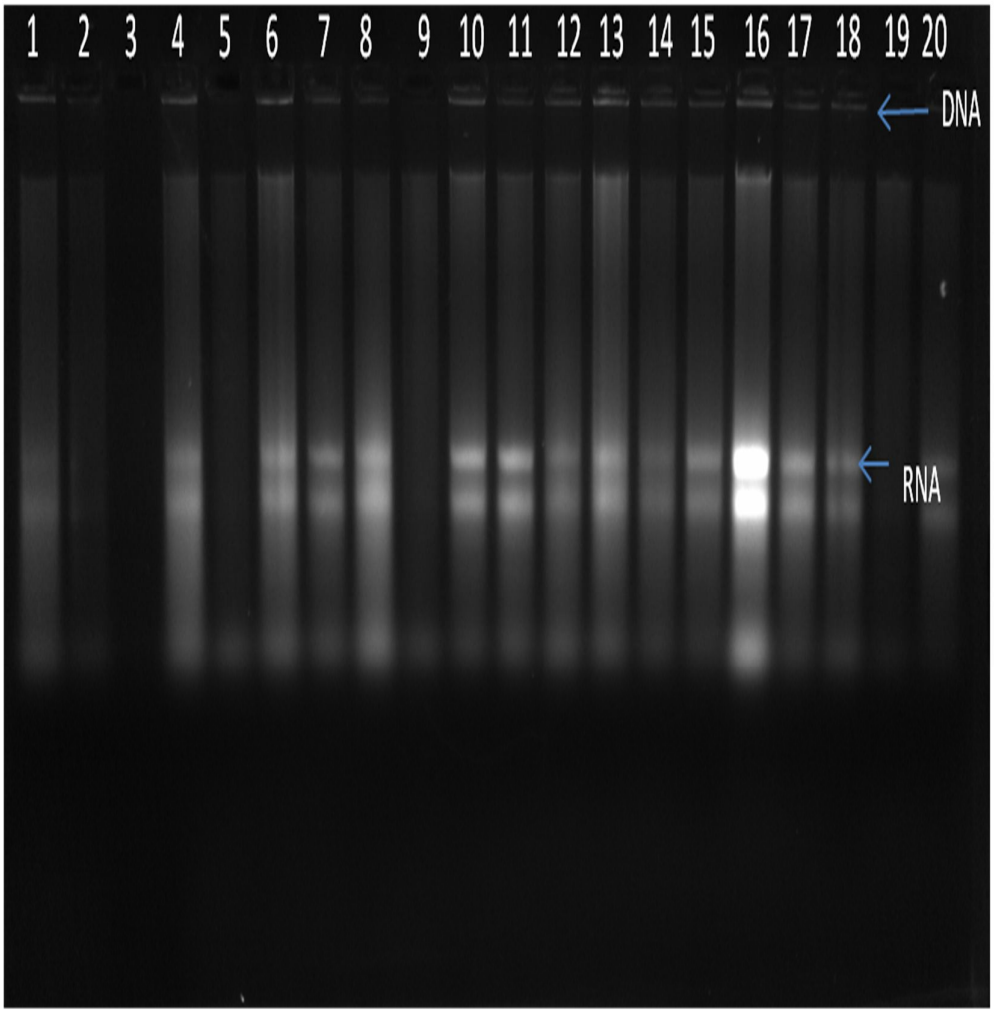


Figure 2: Integrity test for Banana samples

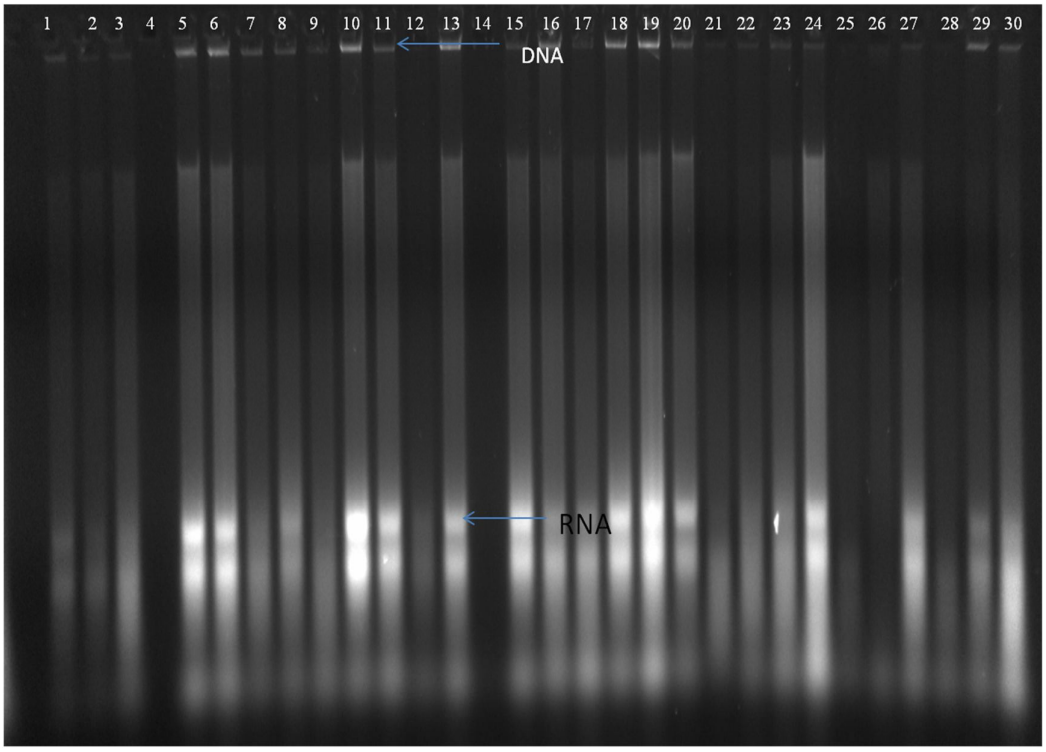


Figure 3: Integrity test for Banana samples

4.5 Determination of DNA/RNA Purity (Nano Drop Test)

The purity and concentration of nucleic acid was measured using Nano Drop spectrophotometer using a number of randomly selected samples as shown in Table 4.4.

4.6 RT-PCR Result for BBrMV in Banana Samples

Detection of BBrMV (RNA viruses) was done by RT-PCR. The RNA of the virus was reversed transcribed into DNA using reverse transcriptase enzyme. The samples analyzed revealed no amplification of virus DNA (Fig. 4)

4.7 RT-PCR Result for CMV in Banana Samples

Detection of CMV (RNA viruses) was carried out using RT-PCR analysis. The RNA of the virus was reversed transcribed into DNA using reverse transcriptase enzyme. Samples that gave faint band for CMV in Fig. 5a and b using 1: 50 dilution were subjected to another RT-PCR analysis by increasing the concentration (1: 20 dilution) and RT-PCR result revealed amplification of virus DNA as shown in Fig. 5c lane 4.

4.8 PCR Result for BBTV in Banana Samples

PCR analysis was used for detection of BBTV (DNA virus). Based on the result, all the *Musa* samples subjected to PCR analysis did not show amplification of viral DNA as show in Fig. 6

4.9 PCR Result for BSV in Banana Samples

PCR analysis for BSV showed 11 out of 49 samples tested had amplification with BSV primer. 7 positive samples from Zaria Local Government (3 from Kamfanin Zango and 4 samples from Faifayi) as shown in Fig. 7a and 4 samples from Igabi local government tested positive for BSV (a sample from Hayin Karma and 3 samples

Table 4.4: Sample Purity using Nano Drop Spectrophotometry

S/N	Nucleic Acid Conc. (ng/µl)	$A_{230\ nm}$	$A_{260\ nm}$	$A_{280\ nm}$	260/280	260/230
1	225.3	2.397	4.507	2.578	1.75	1.88
2	505.1	5.154	10.102	5.685	1.78	1.96
3	286.8	3.169	5.736	3.468	1.65	1.81
4	128.7	2.027	2.574	2.34	1.1	1.27
5	168.4	3.872	3.369	2.912	1.16	0.87
6	216.8	2.332	4.337	2.438	1.78	1.86
7	526.3	5.569	10.526	5.725	1.84	1.89
8	294.8	3.186	5.895	3.618	1.63	1.85
9	582.6	5.915	11.653	6.016	1.94	1.97
10	263.2	3.486	5.264	3.342	1.58	1.51
11	377.1	4.167	7.542	4.096	1.84	1.81
12	226.3	2.332	4.525	2.297	1.97	1.94
13	893	9.867	17.86	9.929	1.8	1.81

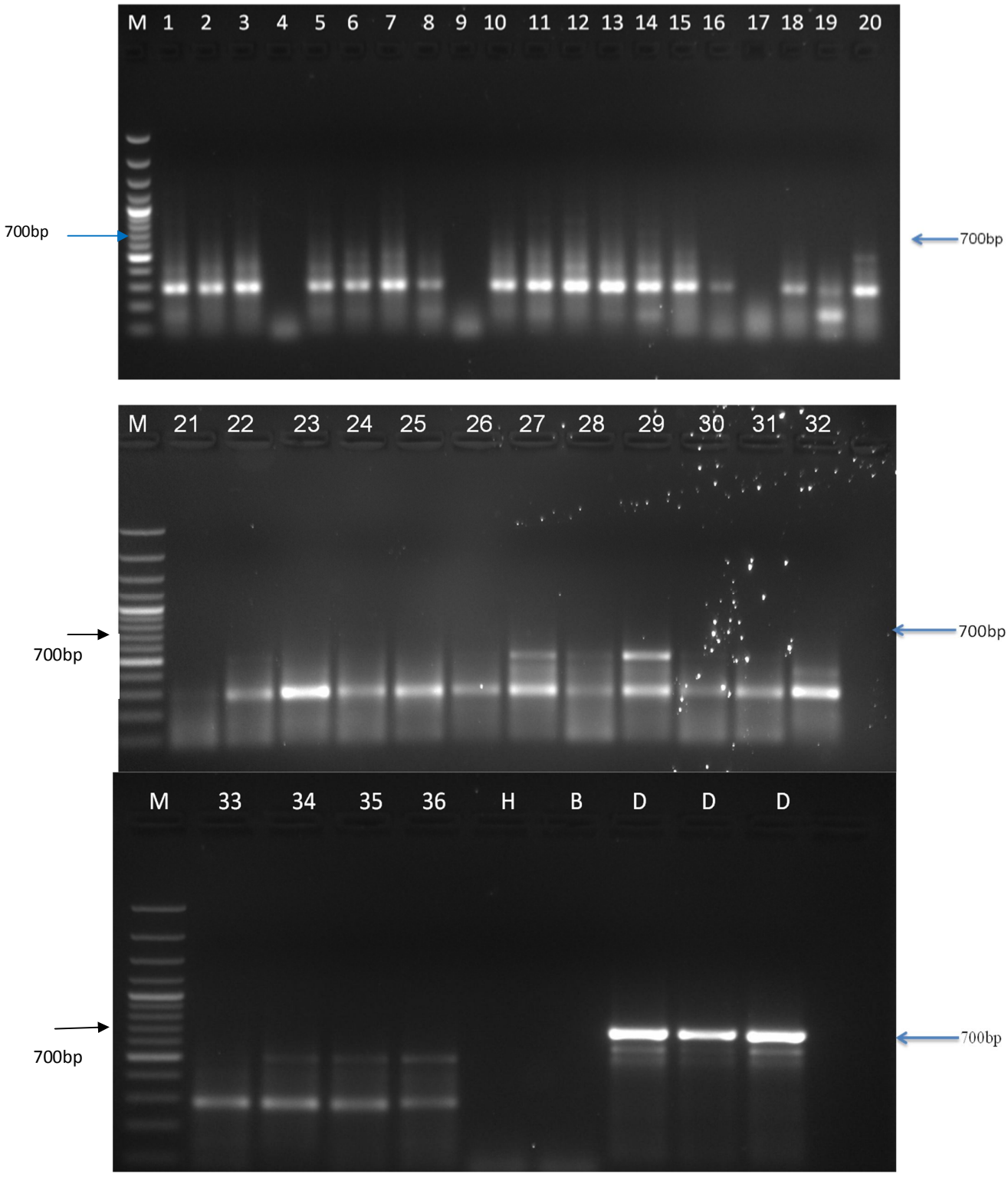


Figure 4: Agarose gel electrophoresis of products from RT-PCR using CI Primer. Lane M - 100bp marker, Lanes 1 to 36- Banana, Lane H – Healthy control, Lane B – Buffer, Lane D – Diseased control (700bp)

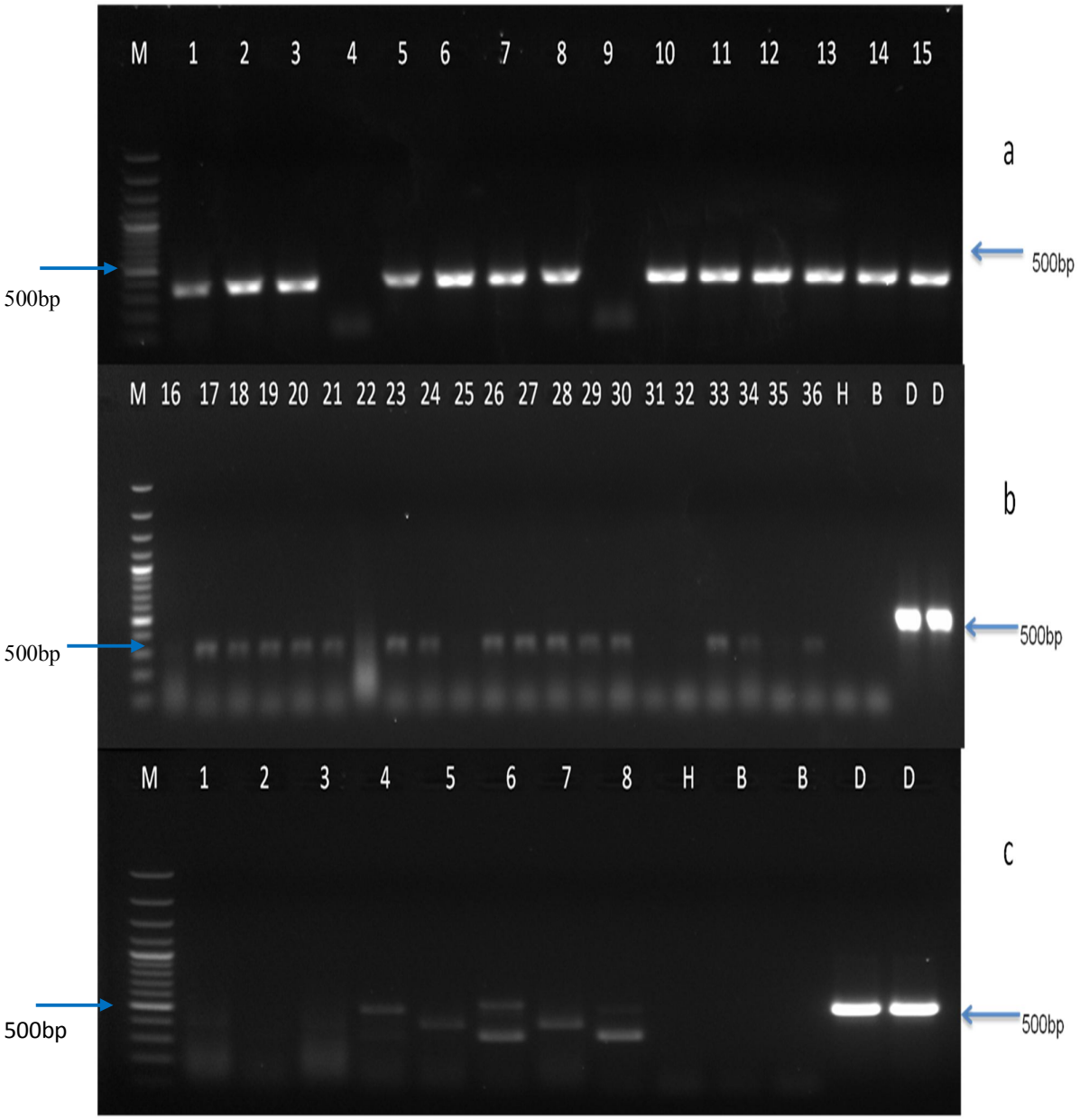


Figure 5: Agarose gel electrophoresis of products from RT-PCR using CMV Primer. M - 100bp marker, Lanes 1 to 40 (fig a and b)- Banana, fig c: Lane 4 (positive CMV) is sample 15 (fig. a) at 1/20 dilution, lane 8 (fig. c) is sample 15 (fig. a) at 1/50 dilution, Lane H – Healthy control, Lane B – Buffer, Lane D – Diseased control (500bp)

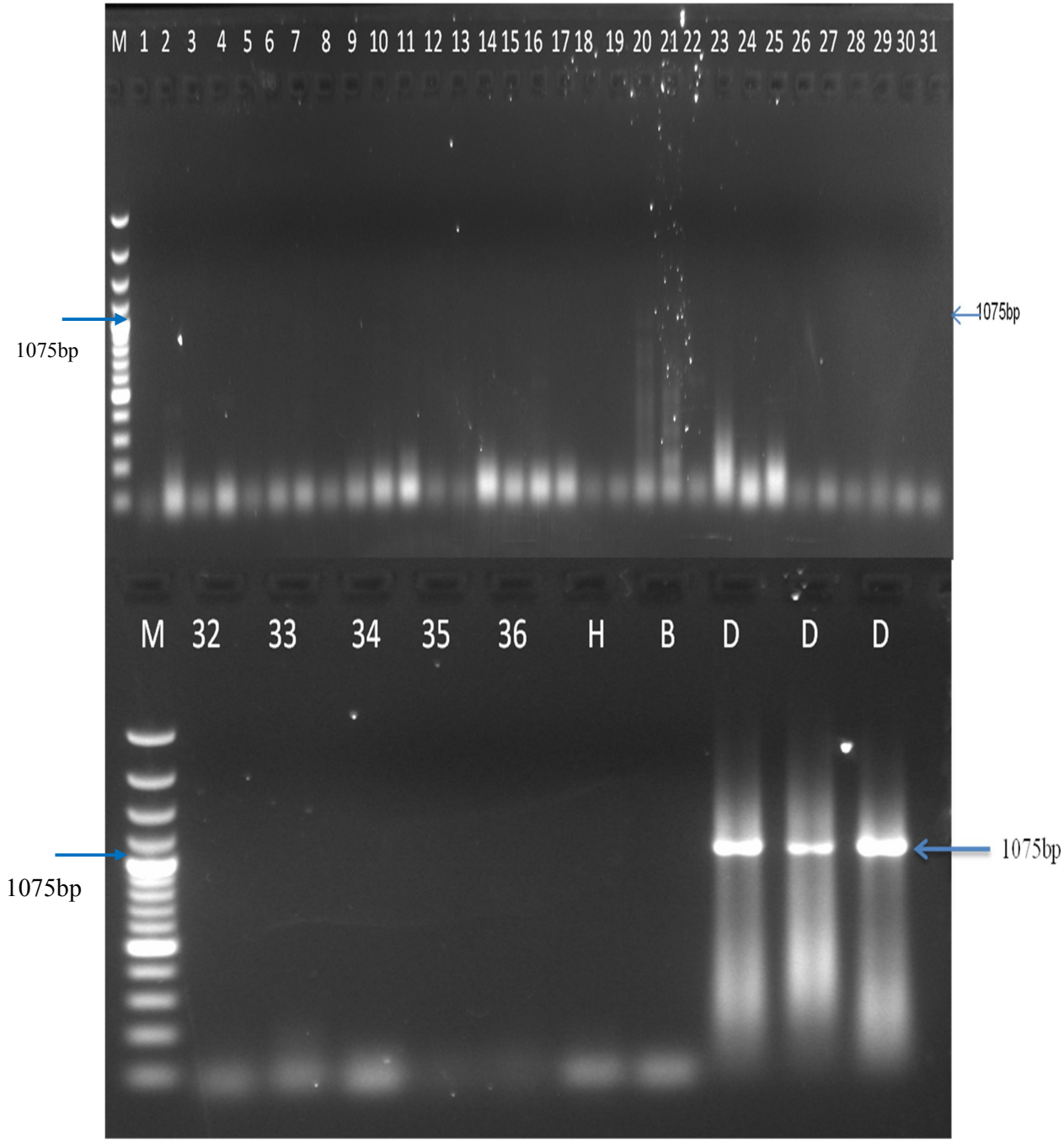


Figure 6: Agarose gel electrophoresis of products from PCR using Scp Primer. Lane M - 100bp marker, Lanes 1 to 36- Banana, Lane H – Healthy control, Lane B – Buffer, Lane D – Diseased control (1075bp)

from Hayin Igugu) as shown in Fig 7b. While all the samples tested in Chukun Local Government did not show amplification for BSV (Fig. 7c).

4.10 Tabular Representation of Symptom Severity from Banana Samples

Table 4.5 shows the symptom severity for banana samples. Symptoms of viral disease on leaf samples were scored on a scale of 0-5 (Karanja, 2010). Samples that were healthy based on symptom expression in the field (samples 6, 9, 10, 11, 13, 28, 30 and 34) did not show amplification for BSV while samples 3, 8, 15, 16, 18, 20, 21, 22, 23, 24, 29, 32, 33, 35, 43, 44, 45, 46, 47, 48 and 49 that were symptomatic in the field did not show amplification for BSV. The distribution of the viruses in the sample sites is shown in fig 8.

4.11 Analysis of Banana Samples for BSV

The result of the analysis shows significant difference for symptom severity as well as PCR result as shown in Appendix 3 and 4. Based on symptom severity, symptoms of BSV disease in Buruku farm is significantly different from the other farms as shown in Table 4.6. In terms of PCR result, Faifayi is significantly different from Kamfanin Zango, Birnin Yero and Buruku farms.

4.12 RT-PCR Result for Potyvirus in Weed Samples

The result showed amplification for potyvirus in 6 weed samples as shown in Plate XII. The sharper the band, the more the concentration of the virus in the sample. *Emilia pratermissa* (Asteraceae) from Hayin Karma, Two samples of *Commelina diffusa* (Commelinaceae) from two different locations in Kamfanin Zango, a sample of *Commelina diffusa* (Commelinaceae) from Birnin Yero, *Hypoestes verticillaria*

(Acanthaceae) and *Cynodon dactylon* (poaceae) from Buruku farm as shown in Table 4.7 were positive for Potyvirus.

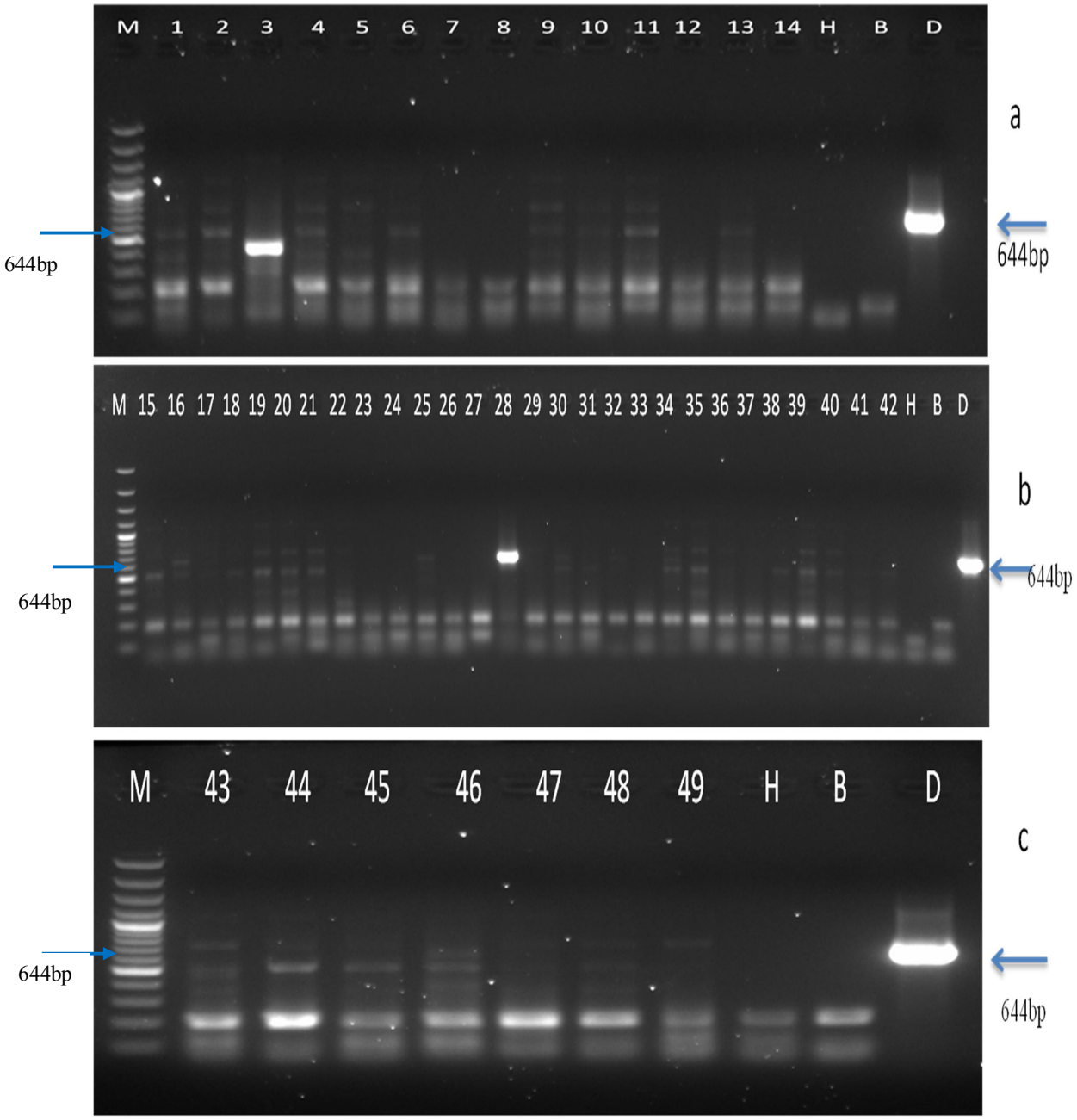


Figure 7: Agarose gel electrophoresis of products from PCR using BSV-Onne Primer, M - 100bp marker, Fig a- Banana from Zaria L.G.A (Lane-2,4,6,9,10,11, 13 were positive), Fig b, Banana from Igabi L.G.A (Lane- 28,30,32,34 are positive), Fig c-

Banana from Chikun L.G.A (Lane 43-49 no amplification), H – Healthy control, B – Buffer, D – Diseased control (644bp)

Table 4.5: Incidence of banana viruses based on symptom severity and PCR result from the studied areas

S/N	Farm	Symptom Severity	RT-PCR BBTV	RT-PCR poty	PCR CMV	PCR BSV
1	Rafin mairabo	0	-	-	-	-
2	Rafin mairabo	2	-	-	-	+
3	Rafin mairabo	5	-	-	-	-
4	Rafin mairabo	1	-	-	-	+
5	Rafin mairabo	0	-	-	-	-
6	Rafin mairabo	0	-	-	-	+
7	Rafin mairabo	0	-	-	-	-
8	Faifayi	3	-	-	-	-
9	Faifayi	0	-	-	-	+
10	Faifayi	0	-	-	-	+
11	Faifayi	0	-	-	-	+
12	Faifayi	0	-	-	-	-
13	Faifayi	0	-	-	-	+
14	Faifayi	0	-	-	-	-
15	Kamfani	2	-	-	+	-
16	Kamfani	4	-	-	-	-
17	Kamfani	0	-	-	-	-
18	Kamfani	3	-	-	-	-
19	Kamfani	0	-	-	-	-
20	Kamfani	2	-	-	-	-
21	Kamfani	1	-	-	-	-
22	Hayin karma	2	-	-	-	-
23	Hayin karma	3	-	-	-	-
24	Hayin karma	1	-	-	-	-
25	Hayin karma	0	-	-	-	
26	Hayin karma	0	-	-	-	-
27	Hayin karma	0	-	-	-	- +
28	Hayin karma	0	-	-	-	
29	Hayin Igugu	2	-	-	-	-
30	Hayin Igugu	0	-	-	-	+
31	Hayin Igugu	0	-	-	-	+
32	Hayin Igugu	3	-	-	-	+
33	Hayin Igugu	4	-	-	-	-
34	Hayin Igugu	0	-	-	-	+
35	Hayin Igugu	4	-	-	-	-
36	Birnin Yero	0	-	-	-	-

37	Birnin Yero	0	-	-	-	-
38	Birnin Yero	0	-	-	-	-
39	Birnin Yero	0	-	-	-	-
40	Birnin Yero	0	-	-	-	-
41	Birnin Yero	0	-	-	-	-
42	Birnin Yero	0	-	-	-	-
43	Buruku	4	-	-	-	-
44	Buruku	4	-	-	-	-
45	Buruku	3	-	-	-	-
46	Buruku	5	-	-	-	-
47	Buruku	4	-	-	-	-
48	Buruku	3	-	-	-	-
49	Buruku	4	-	-	-	-

Key

	Symbol
PCR positive	+
PCR Negative	-

Symptom severity for each sample was scored on a scale of 0-5, modification of Karanja, (2010) (Apendix 2):

0 - No symptoms

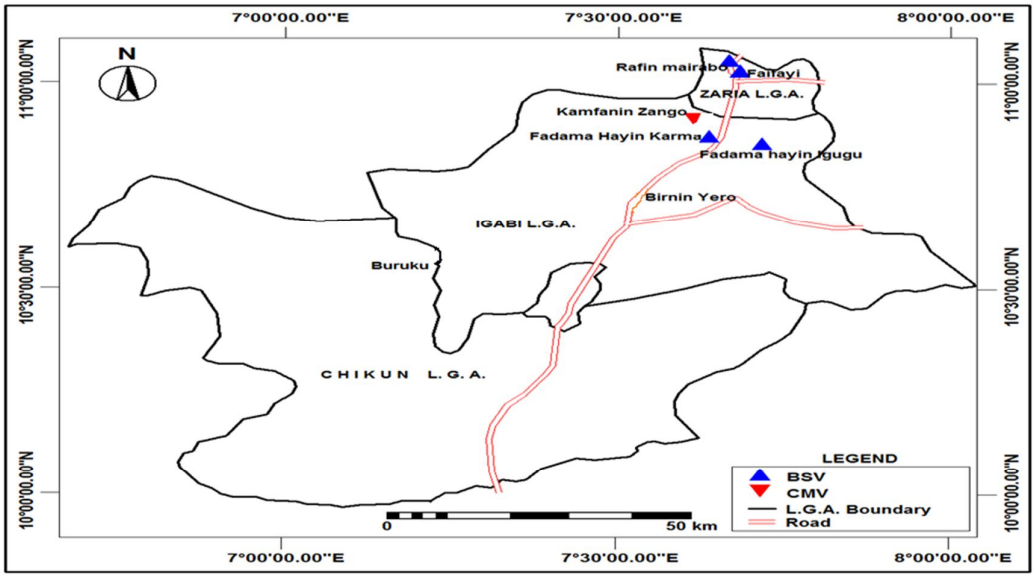
1 - Localised flecks

2 - Scattered discontinuous streaks

3 - Continuous streaks covering moderate portion of lamina

4 - Continuous and conspicuous chlorotic streaks

5 - Necrotic streaks



Source: Adapted and modified from Administrative Map of Kaduna State

Fig 8: Distribution of viruses in the sampling sites

Table 4.6: Mean symptom expression (based on scale 0-5) and PCR result for BSV in the surveyed areas

S/N	Farms	Symptom Severity	PCR Result
1	Rafin mairabo	1.14 ± 0.70^{ab}	0.43 ± 0.20^{ab}
2	Faifayi	0.43 ± 0.43^{ab}	0.57 ± 0.20^{b}
3	Kamfanin Zango	1.71 ± 0.57^{b}	0.00 ± 0.00^{a}
4	Hayin Karma	0.86 ± 0.46^{ab}	0.14 ± 0.14^{ab}
5	Hayin Igugu	1.86 ± 0.70^{b}	0.43 ± 0.20^{ab}
6	Birnin Yero	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
7	Buruku	3.86 ± 0.26^{c}	0.00 ± 0.00^{a}

Means with different superscript within column are significantly different at ($p<0.05$).

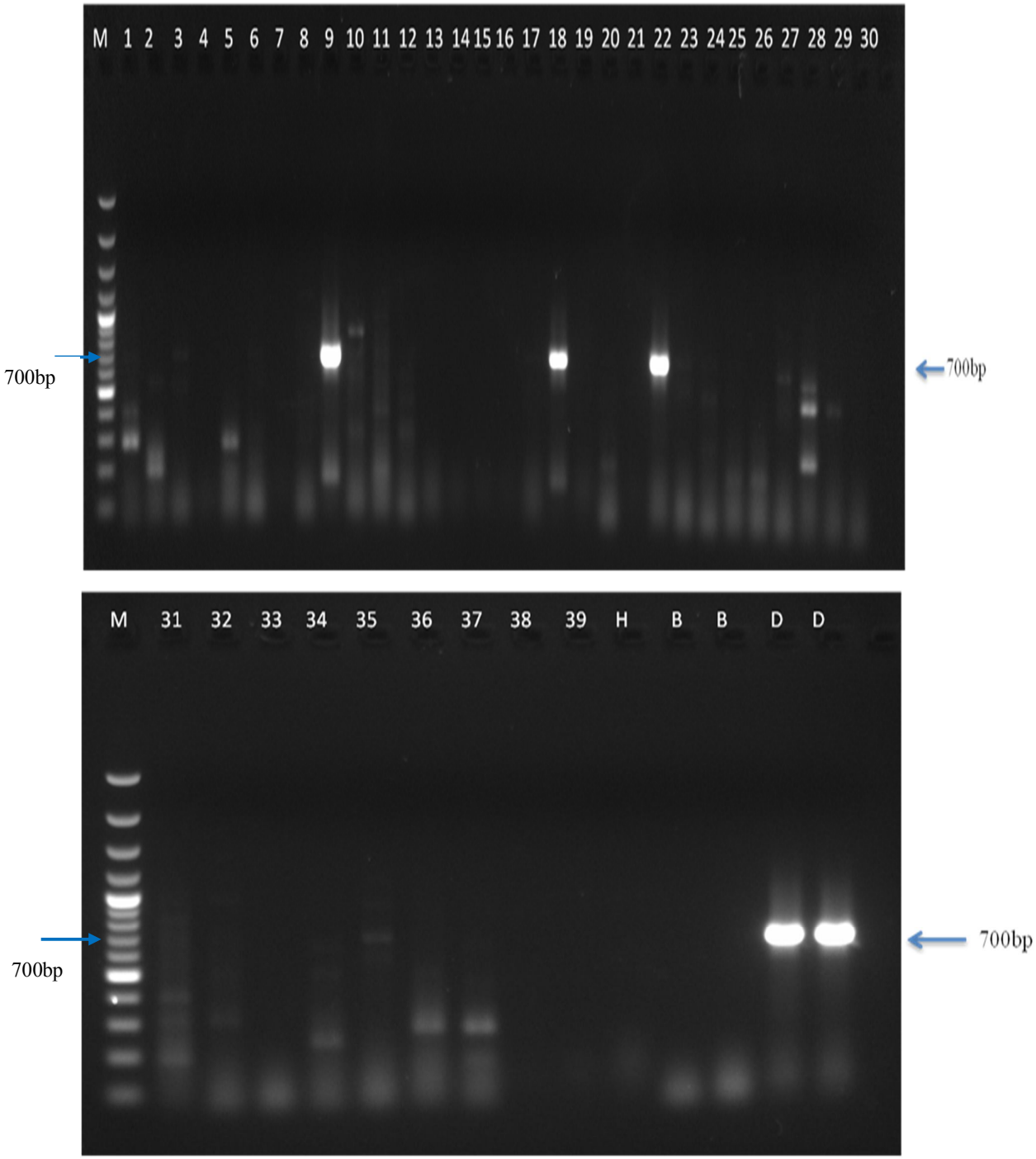


Plate XII: Agarose gel electrophoresis of products from PCR using CI Primer. M - 100bp marker, Lane 1-39 are weed sample, Lanes 3,9,18,22,27,35 are positive, H – Healthy control, B – Buffer, D – Diseased control (700bp).

4.15 RT-PCR Result for CMV Weed Samples

RT-PCR analysis revealed the amplification of a sample out of the 39 samples analysed for CMV as shown in Plate XIII. *Centella asiatica* (Umbellifarae) from Kamfanin Zango out of all the samples was positive for CMV (Table 4.7).

4.16 PCR Result for BSV in Weed Samples

Out of the samples tested for BSV, there was amplification for BSV in 11 samples as shown in Plate XIV. The sharper the band, the more the concentration of the virus in the sample. These samples are; *Clerodendrum* (Verbanaceae) from Rafin Mairabo, five weed samples from Kamfanin Zango: *Nelsonia canescence* (Acanthaceae), *Commelina diffusa* (Commelinaceae) from 3 different locations, *Centella asiatica* (Umbellifarae), *Commelina diffusa* (Commelinaceae) from Birnin Yero and four weed samples from buruku farm: *Hypoestes verticillaria* (Acanthaceae), *Colocasia esculenta* (Araceae), *Cyperus sp* (Cyperaceae), and *Cynodon dactylon* (poaceae) as shown in Table 4.7.

From table 4.7, it can be seen that some weed samples have single or mixed infection of both Potyviruses and BSV. Similarly, all the samples positive for Potyviruses with the exception of *Emilia pratermissa* (Asteraceae) were also positive for BSV indicating that the samples have multiple infections. *Commelina diffusa* from different location were positive for potyvirus and BSV indicating that it is a good reservoir host for the virus.

4.15 Antigen Coated Plate (ACP) ELISA for CMV and Potyvirus Weed Samples

ACP ELISA was carried out on weed samples that were positive for potyvirus and CMV when subjected to RT-PCR. A sample that was positive for CMV with PCR

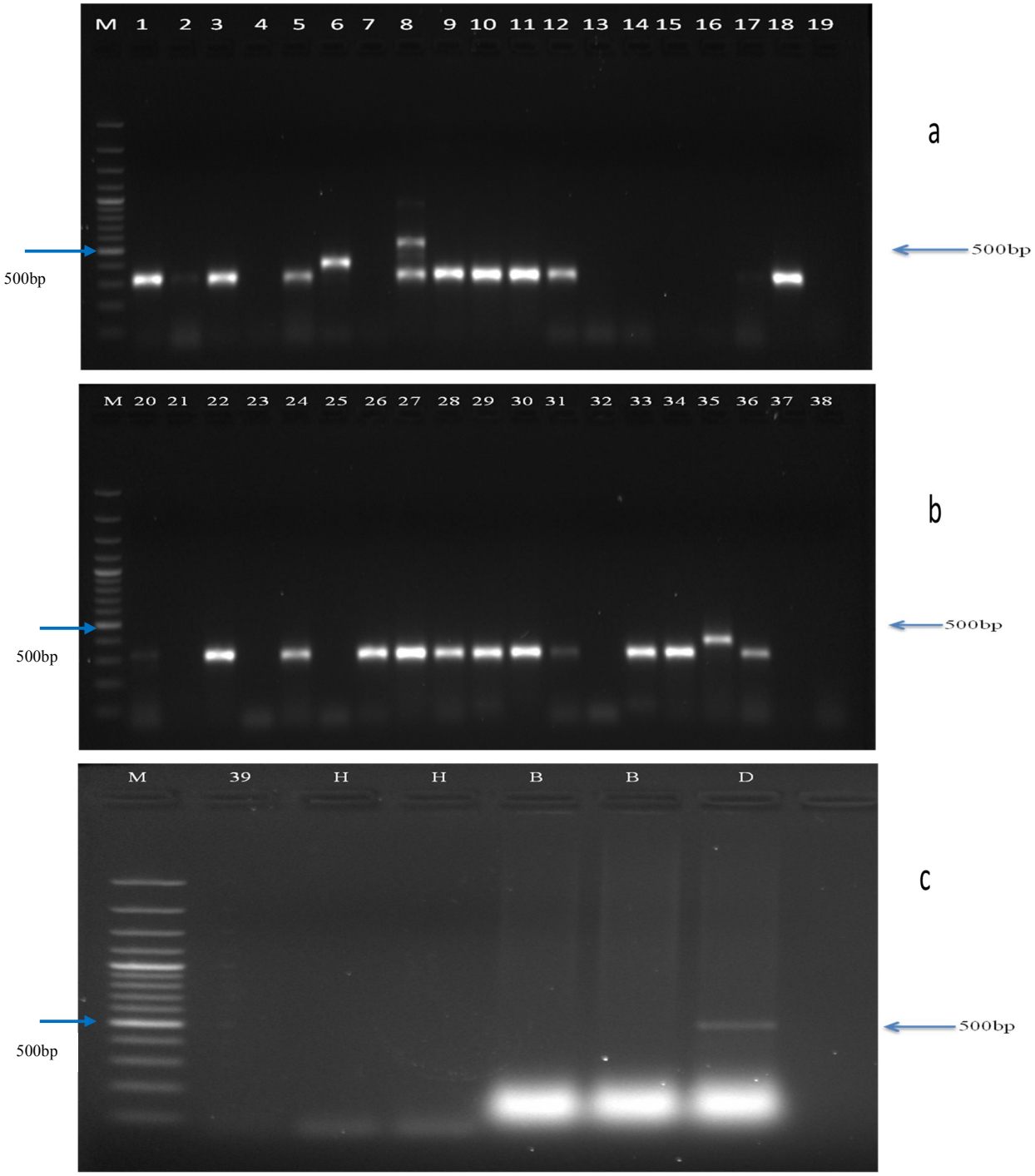


Plate XIII: Agarose gel electrophoresis of products from PCR using CMV Primer. Lane M - 100bp marker, Lanes 1 to 39- weed samples, Lane 8 is positive, Lane H – Healthy control, Lane B – Buffer, Lane D – Diseased control (500bp).

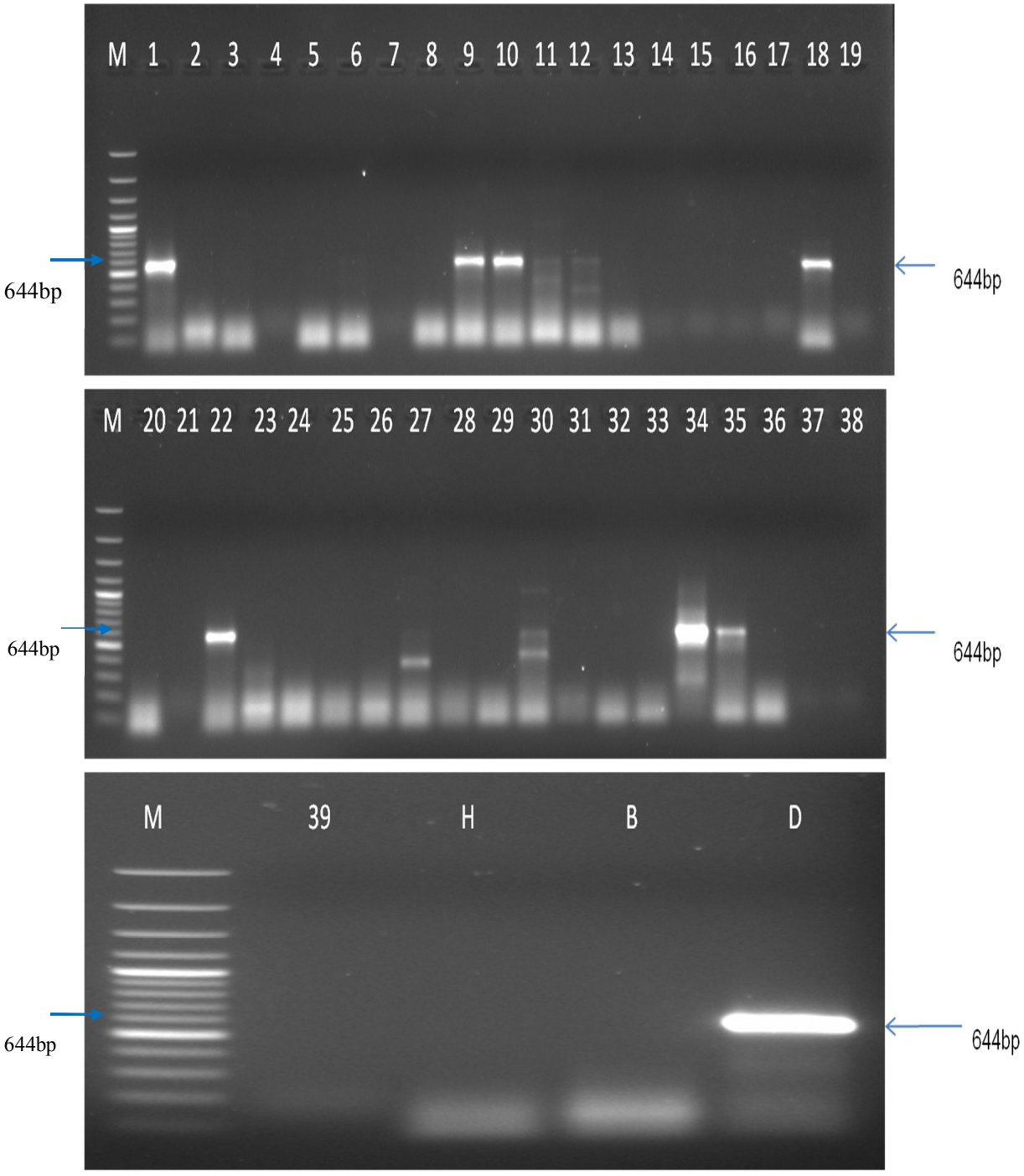


Plate XIV: Agarose gel electrophoresis of products from PCR using BSV-Onne Primer. M - 100bp marker, Lanes 1-39 were weed samples, Lanes 1,9,10,11,12,18,22,27,30,34,35 were positive, H – Healthy control, B – Buffer, D – Diseased control (644bp).

Table 4.7: Weeds subjected to PCR/RT-PCR

S/N	Botanical names	Family	Locations	PCR Poty	PCR CMV	PCR BSV
1	*Clerodendrum* sp (Lam.)	Lamiaceae	Rafin mairabo	-	-	+
2	*Macrospyra longistyla*	Rubiaceae	Rafin mairabo	-	-	-
3	*Emilia pratermissa* (Milne-Redhead)	Asteraceae	Hayin karma	+	-	-
4			Hayin karma	-	-	-
5	*Euphorbia hirta* (L)	Euphorbiaceae	Hayin karma	-	-	-
6	*Sclercia verrucosa* (Willd)	Cyperaceae	Hayin karma	-		-
7			Kamfanin zango	-	-	-
8	*Centella asiatica* (L)	Apiacea	Kamfanin zango	-	+	-
9	*Commelina diffusa* (Burm. f.)	Commelinaceae	Kamfanin zango	+	-	+
10	*Commelina diffusa* (Burm. f.)	Commelinaceae	Kamfanin zango	-	-	+
11	*Centella asiatica* (L)	Apiaceae	Kamfanin zango	-	-	+
12	*Nelsonia canescence* (Lam.)	Acanthaceae	Kamfanin zango	-	-	+
13	*Emilia pratermissa* (Milne-Redhead)	Asteraceae	Kamfanin zango	-	-	-
14	*Ageratum conyzoides* (L)	Asteraceae	Kamfanin zango	-	-	-
15	*Ageratum conyzoides* (L)	Asteracea	Kamfanin zango	-	-	-
16	*Ageratum conyzoides* (L)	Asteraceae	Kamfanin zango	-	-	-
17	*Centella asiatica* (L)	Apiaceae	Kamfanin zango	-	-	-
18	*Commelina diffusa* (Burm. f.)	Commelinaceae	Kamfanin zango	+	-	+
19	*Spicathes pilicansis*		Birnin yero	-	-	-
20	*Cleome* spp (L)	Cleomaceae	Birnin yero	-	-	-
21	*Centella asiatica* (L)	Apiaceae	Birnin yero	-	-	-
22	*Commelina diffusa* (Burm. f.)	Commelinaceae	Birnin yero	+	-	+
23	*Desmodium* spp (Desv)	Fabaceae	Birnin yero	-	-	-
24	*Senna occidentalis* (L) Link	Fabaceae	Birnin yero	-	-	-
25	*Alternanthera sessilis* (L) R.Br. ex DC	Amaranthaceae	Buruku	-	-	-
26	*Euphorbia hirta* (L)	Euphorbiaceae	Buruku	-	-	-
27	*Hypoestes verticillaris* (Linn. f.)	Acanthaceae	Buruku	+	-	+
28			Buruku		-	-
29	*Calopogonium mucoronata*		Buruku	-	-	-
30	*Colocasia esculenta*	Araceae	Buruku	-	-	+
31	*Nelsonia canescens* (Lam.)	Acanthaceae	Buruku	-	-	-
32	*Portulaca olearaceae* (L)	Portulacaceae	Buruku	-	-	-
33	*Euphorbia hyssopitolia* (L)	Euphorbiaceae	Buruku	-	-	-
34	*Cyperus* spp (L)	Cyperaceae	Buruku		-	+
35	*Cynodon dactylon* (L) Pers	Poaceae	Buruku	+	-	+
36	*Euphorbia hirta* (L) Millsp	Euphorbiaceae	Buruku	-	-	-
37			Hayin karma	-	-	-
38	*Panicum maximum* (L)	Poaceae	Rafin mairabo	-	-	-
39	*Asystasia gangetica* (L)	Acanthaceae	Rafin mairabo	-	-	-

Key

	Symbol
PCR positive	+
PCR Negative	-

turned out negative when tested with ACP ELISA. In addition, six samples positive for potyviruses with PCR were subjected to ACP ELISA using universal antibody for potyviruses and polyclonal antibody for two potyviruses (cowpea aphid borne mosaic virus and cowpea black eye mosaic virus). *Commelina diffusa* was highly positive with general potyvirus and slightly positive with cowpea aphid born mosaic virus (CABMV) indicating that the potyvirus present in the sample was CABMV while the other samples were negative for general potyvirus, CABMV and BLCMV (Table 4.8).

4.16 Quality and Purity of Mealybugs DNA Extracted

The quality of the nucleic acid extracted was determined using integrity test. Plate XV shows visible DNA bands. Purity of the DNA was determined using nano drop spectrophotometry (table 4.9). Nano drop test showed high absorbance for the nucleic acids extracted.

4.17 PCR Result for BSV in Mealybugs

PCR on Mealybug did not show amplification of BSV in all the samples (Plate XVI)

4.18 Quality and Purity of Aphid DNA Extracted

The quality of the nucleic acid extracted was determined using integrity test. Plate XVII shows DNA bands. Purity of the DNA was determined using nano drop spectrophotometry (table 4.10). Nano drop result shows high absorbance for the nucleic acids extracted.

4.19 PCR Result for BBTV in Aphid

PCR analysis followed by agarose gel electrophoresis of Aphid DNA did not show amplification of BBTV in all the samples tested as shown in Plate XVIII.

Table 4.8: Antigen Coated Plate Enzyme Linked Immunosorbent Assay (ACP ELISA) for Potyvirus and CMV

Weed samples	Locations	Antibodies used and their absorbance at 450 nm			
		Gen Poty	CABMV	BLCMV	CMV
Hypoestes verticillaria (Linn. f.)	Buruku	0.23	0.12	0.19	1.74
Cynodon dactylon (L) Pers	Buruku	0.23	0.11	0.13	0.59
Centella asiatica (L)	Kamfanin Zango	0.17	0.1	0.11	0.61
Commelina diffusa (Burm. f.)	Birnin Yero	0.23	0.12	0.14	1.09
Emilia pratermissa (Milne-Redhead)	Hayin Karma	0.23	0.13	0.13	1.03
Commelina diffusa (Burm. f.)	Kamfanin Zango	1.41	0.28	0.15	1.24
Commelina diffusa (Burm. f.)	Kamfanin Zango	0.44	0.15	0.15	1.19
Diseased		0.44	0.5	0.75	1.11
Healthy		0.21	0.12	0.13	0.88
Buffer		0.16	0.19	0.12	0.3

Colour code

Healthy
Diseased

CABMV- Cowpea aphid borne mosaic virus

BLCMV- Black eye cowpea mosaic virus

CMV- Cucumber mosaic virus

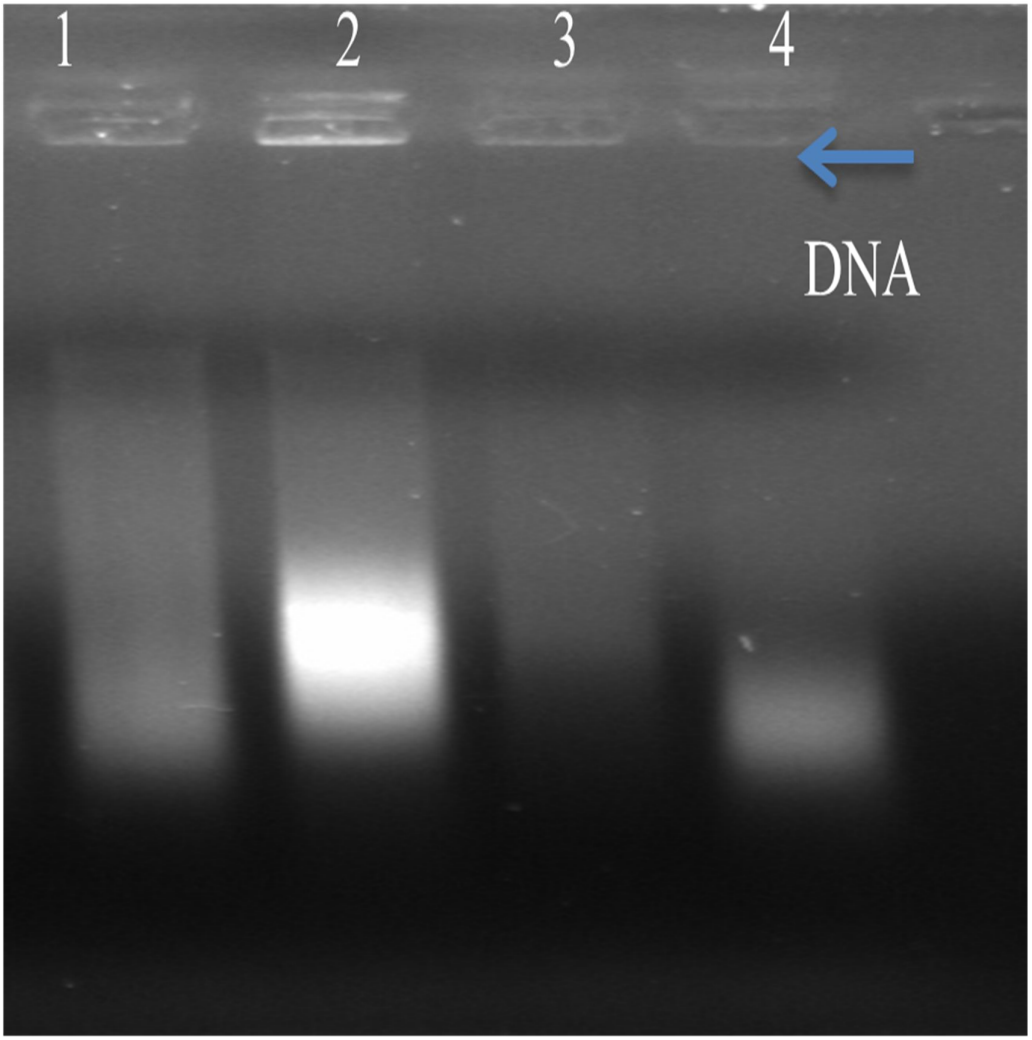


Plate XV: Integrity test for Mealybug

Table 4.9: Nano drop test for some Mealybugs

S/N	Nucleic Acid Conc. (ng/µl)	$A_{230\ nm}$	$A_{260\ nm}$	$A_{280\ nm}$	260/280	260/230
1	263.2	3.486	5.264	3.342	1.58	1.51
2	377.1	4.167	7.542	4.096	1.84	1.81

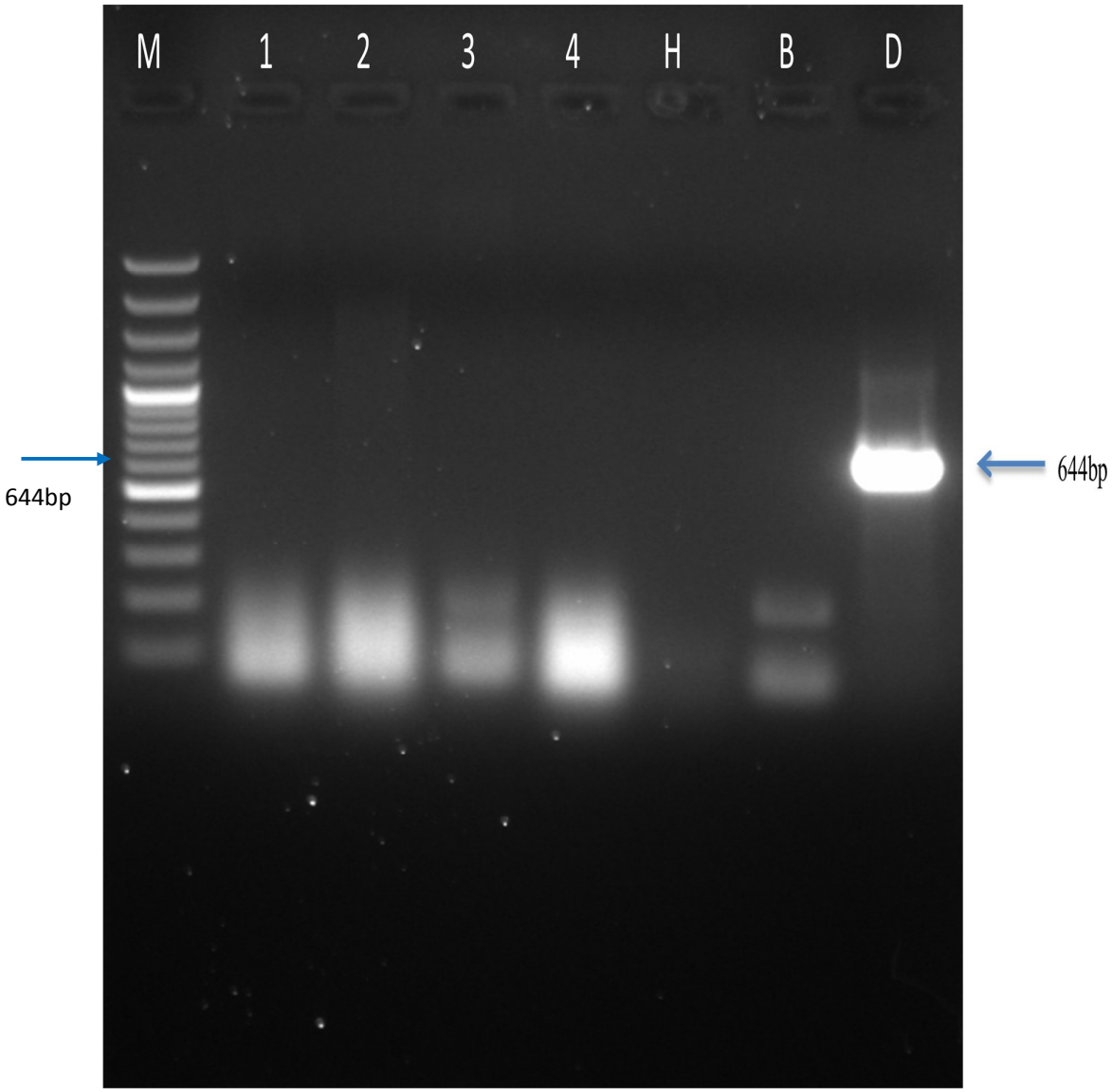


Plate XVI: Agarose gel electrophoresis of products from PCR using BSV-Onne Primer. Lanes 1-4 are Mealybug samples, M - 100bp marker, H – Healthy control, B – Buffer, D – Diseased control (644bp).

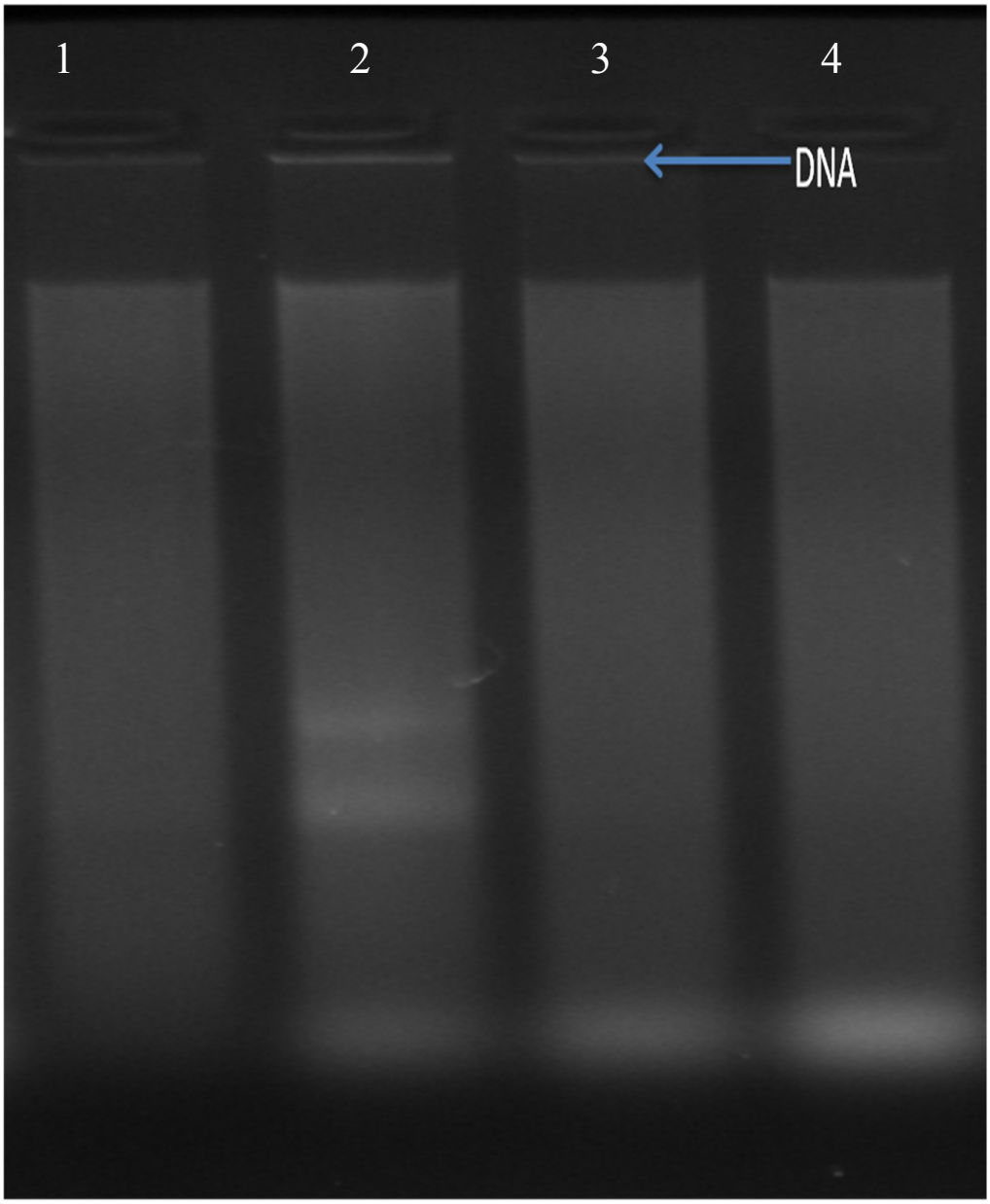


Plate XVII: Integrity test for Aphids

Table 4.10: Nano drop test for some Mealybugs and Aphids Nucleic acid

S/N	Nucleic Acid Conc. (ng/µl)	A_{230nm}	A_{260nm}	A_{280nm}	260/280	260/230
1	226.3	2.332	4.525	2.297	1.97	1.94
2	893	9.867	17.860	9.929	1.8	1.81

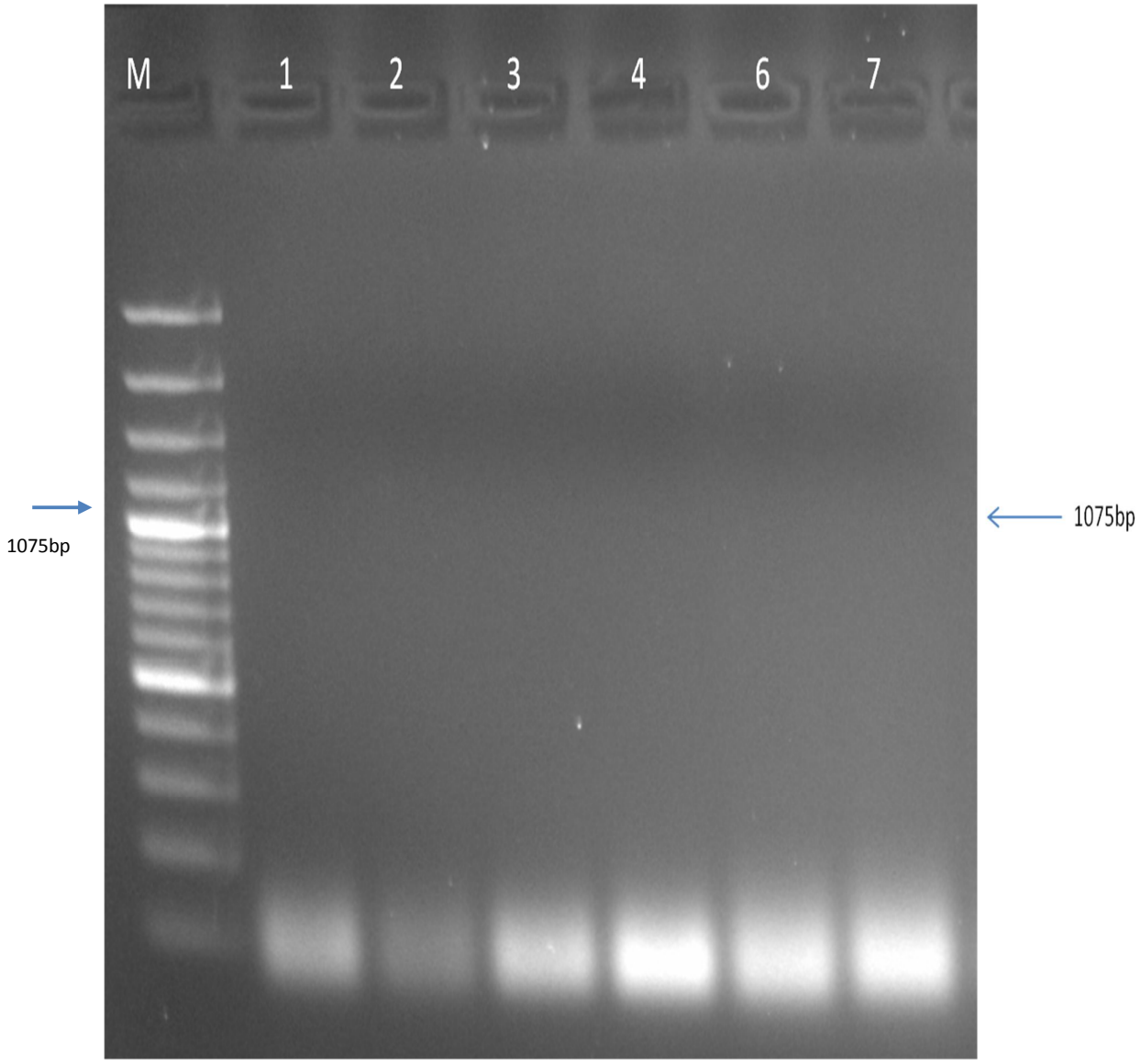


Plate XVIII: Agarose gel electrophoresis of products from PCR using Scp Primer.

Lane M - 100bp marker, Lanes 1 to 36- banana samples, Lane H – Healthy control, Lane B – Buffer, Lane D – Diseased control (1075bp coat protein Scp)

4.20 Other Problems Associated with Banana Cultivation in the Studied Areas

Other problems that were observed in the field during the survey that were not associated to banana viruses are fungal leaf blight (Appendix 5). Another problem is pests. The insect pest affecting banana leaves was the grasshoppers (*Zonocerus variegatus* Linn) which feed on the entire leaf lamina leaving out just the mid rib (Appendix 6). Damage done on banana fruits by rodents was observed (Appendix 7).

A Bush fire is one of the problems that affects banana cultivation in the areas studied resulting in burning of all or almost all banana plantation (Appendix 8a and 8b). One major problem militating against the production and productivity of banana in the study areas is theft. Theft was reported to be the major problem affecting banana cultivation in the three L.G.As visited. Most of banana farmers complained bitterly of theft especially when the fruit is almost ripe. This had made some farmers switched to the cultivation of other crops like sugarcane, tomato, pepper etc. A farmer in Kwanan Farakwai reported that few years back he earns a lot of money from banana cultivation which enable him to feed and clothe his entire family during festivities. But due to theft, he doesn't earn as much now. He also reported that they paint their banana (Appendix 9a and 9b) so that once stolen it can easily be detected in nearby market and retrieved.

CHAPTER FIVE

5.0 DISCUSSION

In all the areas studied, bananas were grown mainly in fadama areas where there is ample supply of water and the environment is moist. However with peasant farmers, bananas were grown mixed with other crops. Bananas were grown as hedge around sugarcane, pepper, onion, tomato and carrot farms. In some of the areas for example, Kamfanin Zango farmers reported that they had orchards but due to theft, they shifted to mixed cropping. Robinson (1996) reported that banana are grown in all types of agricultural systems ranging from small, mixed subsistence gardens to large multinational commercial monocultures. Despite the relegation of growing banana as hedge, farmers still get high yield which cater for some of their financial needs. This indicates that if given technical and financial supports, banana cultivation in the areas will give high boost. One of the advantages is that the husbandry of the banana grown with other crops under irrigation also serves the banana plants as such they have little input directly on the cultivation of banana in terms of manure, water etc. Similar report was given by Karamura *et al*. (1999) that banana plants in eastern and southern Africa were cultivated in combination with traditional food crops on smallholdings of <1 ha.

Two types of banana were grown in the areas surveyed. These are: dessert banana and a tall dessert banana variety (Iyar Lagos). 86% of the farms grow only dessert banana (AAA genome) while 14% grow both banana (AAA genome) and plantain (AAB genome). This is probably because bananas are consumed locally as dessert without being processed as compared with plantain. This observation confirms the report of FAO (2003) that dessert bananas comprised 59% of annual world production while

the cooking bananas (plantain) comprised 17%. On the source of suckers for planting, these were obtained from suckers of old banana plants in the same field. Origin of the first planting material was not gathered because the farmers said that the banana cultivation in those areas was for over 15 years. It is difficult to ascertain the origin of the first suckers even though wild banana were reported to exist in the areas.

During the survey, both symptoms of BSV and CMV were observed on mainly desert banana plants. However, the farmers were ignorant that the symptoms were caused by viral disease, rather, they associated the symptoms with water stress, overcrowding and insufficient fertiliser/manure. Lockhart (2013) reported that farmers could accurately describe the symptoms of BSV and CMV on banana, but tend to associate them with a number of abiotic factors such as poor soils, drought and weevil infestation rather than with a viral cause. Because of the poor perception of symptoms induced by viral diseases, little or no strategic measures were adopted in curtailing the disease. As such, diseases are being spread with planting suckers because it is a vegetatively propagated crop.

The conspicuous symptoms of BSV observed in the areas surveyed agreed with the findings of Jones and Lockhart (1993) who reported a range of symptoms induced by BSV infected plants. These includes: splitting of the outer leaf sheaths of the pseudostem, constriction of the bunch on emergence i.e. choking and abnormal emergence of the bunch from the middle of the pseudostem, bearing fewer fruits of smaller size, yellow streaking or mosaic patterns on leaves, rotting of heart leaves and the centre of pseudo stem i.e. heart rot or sheath rot . These symptoms were observed in all the areas with the exception of Birnin Yero and Buruku farms. However, the heart rot was more conspicuous in Rafin Mairabo farm. In one of the farms (Rafin Mairabo), the farmers reported that they cut open the pseudostem so that the peduncle

can emerge. It was observed that even if the peduncle emerges, it becomes twisted. Similarly, Lockhart and Jones (2000) reported that banana Streak Virus-infected Banana plants frequently express broken or continuous chlorotic or necrotic streaks on the leaves, stunting of diseased plants and occasionally heart-rot of the pseudostem and plant death. Daniells *et al*. (2001) also reported that BSV in some instances causes bunches to burst out midway up the pseudostem.

The symptoms of CMV observed during the survey agrees with the work of Dheepa and Paranjothi, (2010) who reported that CMV causes chlorosis, mosaic as well as heart rot in banana and has been found in most banana growing areas of the world.

Aphids (*Pentalonia* sp) which have been reported to be vectors of BBTV, BBrMV and *Myzus persicae* (Sulzer) were noticed during the survey. Aphids were not amplified using primers for BBrMV and CMV because these viruses are transmitted non persistently by aphid vector and transmit the disease during brief feeding (Palukaitis and Garcia-Arenal, 2003). Robinson, (1996) noted that banana aphid (*Pentalonia nigronervosa* (Coquerel)) was widely distributed in all banana-growing areas. Also during the survey, mealybugs (*Planococcus* sp) were observed on leaves of banana. Root mealybugs (*Dysmococccus* sp) were also noticed. These mealybugs have been reported to be vectors of BSV (Lockhart, 1993 and Kubiriba *et al*., 2001b). Even though attempt to track mealybugs on the corm of banana fail during this study, however the mere presence of mealybugs on the root of some weeds indicate that they could be possible vectors of the viruses in these areas. Similar report was given by Kubiriba *et* al. (2001a) in Uganda who was able to track the mealybugs (*Dysmococccus brevipes* (Cockerell)) in the root and corm of banana and associated it with BSV transmission.

As common with other cultivated crops, both broad and narrow leaf weeds were noticed growing around banana farms in the areas studied. mealybugs and aphids were seen on some weeds like *Cyperus* sp and *Commelina diffusa* (Burm.f.) respectively in Buruku farm on both aerial as well as the roots of weeds. The occurrence of these insects on weeds coupled with the symptoms observed on some weeds will influence the ecology and epidemiology of BSV and CMV in the areas. Because of the theft problem, farmers lose confidence in the banana cultivation and they rarely weed their farms which encourages colonisation by rodents as well as spread of bush fire during dry season as observed in Rafin Mairabo and Kamfanin Zango. Robinson, (1996) reported that the occurrence of weeds in West Africa is major constraint in the production of banana crop for subsistence farmers because they lack effective weed control measures leading to overall yield decline.

On the incidence of banana viral diseases in the areas studied, conspicuous symptoms of BSV and CMV were observed. However, symptos of BBTV and BBrMV were not observed. Report on incidence of BSV have been documented in many Afrian countries For example, Lasoudiere (1974) reported BSV incidences of 6–53% in Côte d'Ivoire, Kubiriba *et al*. (1999) reported 2–100% BSV incidence in Uganda, while Karanja *et al*. (2010) reported 81% incidence for BSV. On a worldwide scale, banana streak virus has been reported to be widely distributed in banana (Lockhart and Olszewski, 1993; Kubiriba and Tushemereirwe, 1999). The later also added that in terms of incidence, CMV follows after BSV in importance. This is the first report of incidence of BSV (29%) in banana in the northern guinea savanna of Nigeria.

Incidence of CMV was low in the areas surveyed with only 16% incidence. This is probably because CMV is not as widely distributed as BSV on banana. The low incidence of CMV observed in banana in the study areas agrees with the work of

Kubiriba and Tushemereirwe, (1999) who reported very low incidence in South Africa. Similarly, Karanja (2010) also reported low CMV incidence of 6% infection on some of the cultivars banana cultivars in Kenya.

Based on symptomatology, BBTV and BBrMV were not observed in the areas surved probably because the viruses are not as widely distributed as BSV and CMV. Similar report were made from Kenya where their was 0% incidence of BBTV and BBrMV (Karanja (2010).

Other earlier studies made by Kenyon *et al.* (1997) in Malawi reported BBTV as being less distributed than BSV. Kubiriba and Tushemereirwe, (1999) reported BBTV to have less incidence in Nigeria although its occurrence was recorded in a number of countries. Niyongere *et al*., (2013) reported that BBTV incidence varied among banana cultivars and locations.

From the values obtained in this study, the absorbance ratio A_{260}/A_{280} was greater than 1.7. Also the ratio of A_{260}/A_{230} was greater than 1.5 for most of the samples. This shows that the samples were pure and sufficient for PCR/RT-PCR. Olswald, (2007) reported that DNA absorbs light strongly at 260 nm so the absorbance value at this wavelegth (called A_{260}) can be used to estimate the DNA concentration and 280 nm is used as an indication of protein contaminant since tyrosine and tryptophan residues absorb strongly at this wavelength. Also A_{230} nm provides a general measurement of the turbidity of the sample and phenolic contaminants absorb at that wavelength. He futher stated that a good quality DNA sample should have A_{260}/A_{280} ratio of 1.7-2.0 and an A_{260}/A_{230} ratio of greater than 1.5 which serves as a guide to the purity of the sample. Similarly, Wambulwa (2013) reported $A_{260/280}$ of 1.8-2.0 which implied that the DNA is pure and sufficient enough for PCR while lower values indicate presence

of protein contaminants and values greater than 2.0 imply that the DNA extract is contaminated with polyphenols.

The selected samples for Integrity test showed visible bands for DNA and RNA. Olswald, (2007) reported that agarose gel is the best method for determination of DNA quality than spectrophotometric measurement since genomic DNA, RNA, guanidium and protein contaminants all display absorbance at 260 nm with nano drop. If contaminants are present at high level in DNA preparation, it will lead to increase in A_{260}nm reading and an over estimation of DNA concentration. He stated that agarose can be used as an alternative approach to measure sample DNA concentration which is not affected by this contaminants.

The result of the PCR analysis for the samples showed that BSV is the major disease problem of banana in the studied areas because it was detected in almost all the farms with the exception of Birnin Yero farm. CMV follows BSV with only a sample from Kamfanin Zango that showed amplification for CMV.

The result of this study agrees with the work of Kubiriba and Tushemereirwe, (1999) who reported that BSV is the most widely distributed and also most important in terms of incidence and CMV follows after BSV in importance. The high occurrence of BSV is in line with the experiment conducted by Gauhl *et al.,* (1999) in the plantain-growing area of southern Nigeria and Wambulwa (2011) in Kenya who observed that BSV is common and widespread. However, the detection of CMV did not agree with the finding of Gauhl *et al,* (1999) in Nigeria and Wambulwa (2012) in Kenya where samples exhibiting BSV symptoms were indexed both for BSV and CMV and most of the samples tested positive for BSV while no CMV was detected.

Among the samples positive for BSV were symptomless plants from Rafin Mairabo, Faifayi, Hayin Karma and Hayin Igugu. This points out clearly that the virus is not only detected in symptomatic plants but also in asymptomatic plants. This may probably be due to the fact that the virus was in a latent stage or the viral symptoms were not expressed due to high temperature when the samples were collected. Similar report was given by Dahal *et al.* (1999) in an experimental field in Nigeria established with suckers from symptomatic plantain plants, indexing of leaf samples from both symptomatic and asymptomatic mats resulted in positive tests for 70% of the symptomatic samples and 15% for the asymptomatic samples. This is also in agreement with Daniells *et al*. (2001) who reported that foliar symptoms are periodic in expression. In a study he conducted, only 45-51% of the leaves produced in a season showed symptoms and infected plants can be symptomless for long periods. He further added that greater symptom expressions were observed in cooler wet season compared with the hot dry season. Karanja *et al.* (2008) also reported that severe and moderate streaked symptom leaf turned out to be negative by direct virus PCR. Agindotan *et al.* (2006) reported that it still remains unknown which, and how many, genomic variants are regulating the symptom expression under certain environmental conditions and whether observed asymptomatic infected plants may be indicative of the presence of special genomic virus variants.

Some of the symptomatic samples observed in all the farms showed no amplification with PCR analysis. Samples from Rafin Mairabo and Buruku with necrotic symptom at an advanced stage of BSV infection showed no amplification. This is probably because BSV Onne primer was used which detects only BSV Onne isolate and BSV has about 7 isolates (Geering *et al*., 2000; Harper *et al*., 2005; Hull *et al*., 2005; Lheureux *et al*., 2007). Based on sequence information, a number of distinct and BSV

sequences have been reported from banana. Initially, isolates were referred to as Banana streak virus isolates differentiated based on their location or their host cultivar. For example, Banana streak Onne reported by Harper and Hull (1998) was originally collected at International Institute of Tropical Agriculture (IITA) Onne field station in Nigeria. Banana streak Onne later came to be known as Banana streak virus strain OL (BSV-OL). After a sequence identical to the episomal form was later characterised from the nuclear genome of banana cultivar Obino 1' Ewai (Geering *et al*., 2001) and was nearly identical to an isolate from Australia named BSV-RD, or 'Red Dacca' after the host cultivar studied (Geering *et al*., 2000). Based on these isolates, it is suspected that more than one isolate of BSV occurs in these areas because some symptomatic samples could not be amplified using BSV Onne primers. Hence more primers should be used in other to ascertain the nature of the other isolates in the studied areas.

Other BSV reported are Banana streak Mysore virus (BSMyV) and Banana streak GF virus (BSGFV) (Hull *et al*., 2005). A third isolate, Banana streak acuminata Vietnam virus (BSAcVNV), has also recently been proposed based on full-length sequence analyses (Lheureux *et al*., 2007). Although partial sequences have been reported for several other putative virus species, including Banana streak Cavendish virus (BSCavV), (Geering *et al*., 2000), Banana streak Imové virus (BSImV) and Banana streak Uganda A- to -M viruses (Harper *et al*., 2005), the taxonomic status of these viruses remains unresolved (James *et al*., 2011). In 1974, Lassoudiere noted that symptoms expression vary with some isolates and location. However, the disease symptom varies and is thought to depend upon a number of factors such as virus isolate, host genotype, level of management and environmental conditions (Lockhart and Jones, 2000). Karanja *et al.,* (2008) also reported that different types of isolates or

viral DNA concentration might have caused low detection of BSV and that Mysore isolate appeared to elicit more severe BSV symptoms. According to Agindotan *et al.* (2006) the high sensitivity of PCR could not be utilized when PCR was applied to highly variable virus populations found with BSV-like viruses, neither within one region nor in different geographical regions. The BSV-Onne primers used could not amplify all BSV isolates present in the samples tested. Those that were positive indicate that there were variants of BSV Onne in those samples He added that there is high variability of the badnavirus variants inducing leaf streak symptoms of banana. Wambulwa *et al*. (2013) reported that, there is little, contradictory information on the virulence of the various BSV isolates in different banana genotypes and the degree to which symptoms would be expressed. Some of the sharp bands detected on the agarose gel indicate higher concentration of the virus. These agrees with the finding of Dahal *et al.* (1998) who stated that intermittent symptom expression was correlated with virus titre in the leaves with symptomatic leaves containing higher levels of virus. Statistically, the result shows that there was significant difference ($p<0.05$) for BSV symptom severity as well as PCR result for BSV indicating that both vary with location.

The result did not show amplification for BBrMV which agrees with the work of Wambulwa (2011) where there was no detection of BBrMV in all the 56 samples tested. Samples tested for BBTV showed no amplification. This is probably because BBTV is not as widely distributed as BSV and CMV. BBTV was first reported in Nigeria by Adegbola *et al*. (2013).

The weeds samples subjected to PCR analysis showed amplification for BSV, CMV, and Potyvirus. Some weed samples showed single or multiple infections of Potyviruses and BSV. *Commelina diffusa* (Burm f.) from different location were

positive for potyvirus and BSV indicating that it is a good reservoir host for the virus. *Centella asiatica* (L.) was the only weed sample positive for CMV. Lockhart, (2013) reported that CMV transmission is predominately from alternate hosts such as weeds (e.g. *Commelina* spp.) and crops (e.g. cucurbits, tomato). The occurrence of CMV has been reported from many hosts such as Egyptian henbane (Samad *et al*., 2000), *gladiolus* (Raj *et al.,* 2002), *Lycopersicum esculentum* (L.) (Sudhakar *et al*., 2006), Geranium (Verma *et al.,* 2006), *Rauvolfia serpentina* (L.) *and Jatropha curcas* (L.) (Raj *et al*., 2007, 2008).

The detection of BSV on weed samples belonging to family Verbanaceae, Apiaceae, Commelinaceae*,* Acanthaceae*,* Araceae, Cyperaceae and Poaceae does not agree with the findings of Lockhart and Jones (1999) who reported that BSV is transmitted only to plants within the family Musaceae using mealybug vectors.

Detection of potyvirus in some weeds indicates that it is a good reservoir host for the viruses. Ward *et al.* (1994) reported that the genus potyvirus is apparently almost as ancient as flowering plants and has a worldwide distribution throughout higher plants and that it is one of the most successful groups of plant pathogens in the world. The detection of BSV, CMV and Potyvirus in weeds indicates that they serve as reservoir host of the viruses which serve as a potential threat to banana production. This is the first report of BSV, CMV and potyvirus detection in weeds in the areas studied.

Banana and weed samples that showed amplification for CMV and potyvirus with PCR were subjected to ACP ELISA to confirm the potyvirus present because a universal potyvirus primer was used for PCR. Antisera for cowpea aphid borne mosaic virus and black eye cowpea mosaic virus were used to determine the specific potyvirus present. ACP ELISA revealed low detection of the virus as compared with

PCR. None of the samples that showed amplification with CMV primer were positive with ACP ELISA. Only *Commelina diffusa* (Burm f.) form Kamfanin Zango was positive for postyvirus as well as cowpea aphid borne mosaic virus. This indicates that the poty virus present is cowpea aphid borne mosaic virus. The poor detection of viruses with ELISA is in conformity with Harper *et al.* (2002) were he used PCR and ELISA to study field samples of banana streak like symptoms from 14 regions in Uganda. PCR with degenerate primers proved a better detection method than ELISA. Mansoor *et al.,* (2005) reported that PCR as an enzymatic procedure was used successfully to detect very low amounts of nucleic acid belonging to several plant viruses with high sensitivity and specificity. Similar report was given by Sadik *et al*., (2012) that PCR technique has been used successfully to detect very low amounts of nucleic acids.

Even though mealybugs were found on some plants like *Hypoestes verticillaria* (Linn. f.) and *Cyperus* sp which were positive for BSV, mealybug samples from the different locations did not show amplification for BSV indicating that the mealybugs might have not acquired the virus or they may contain other isolates of BSV. Karanja *et al.* (2009) also reported that there was no indication of vector (*Planoccocus* sp.) spread of the BSV virus in all the banana cultivars tested. Similarly, Dahal *et al.* (1998) reported that the main means of transmission of BSV in Nigeria appears to be through dissemination, by farmers, of BSV-infected suckers. Water stress and sustained cooler temperatures seem to be the cause of localized 'outbreaks' of symptoms. Controlled transmission experiments have so far indicated that nymphs of a *Planococcus* species and *Dysmicoccus brevipes* (Cockrell) cultured from banana on pumpkin fruits are capable of transmitting a strain of BSV from banana to banana (Dahal *et al.* 1998). Geering *et al*. (2000) revealed that there are at least 12 different strains or species of

BSV Badnavirus infecting banana in Uganda, and it is possible that these have different vector species specificities. In all, twenty mealybug species were included in the identification key as having been collected from *Musa* plants in Uganda, or from there being reference to their collection from *Musa* plants elsewhere in Africa. The four mealybug genera most often collected from banana plants in Uganda were a *Dysmicoccus* sp. (identified as a putative vector of BSV by Kubiriba *et al*., 2001a), a *Planococcus* sp. (*Pl. citri* was identified as a vector of BSV in the laboratory by Lockhart and Olszewski (1993)) a *Paracoccus* sp. and a *Pseudococcus* sp. (*P comstocki* (Kuwana) was identified as a vector of BSV in a laboratory in Taiwan by Su (1998)).

The result revealed no amplification in aphids tested for BBTV. This tallied with the inability to detect BBTV symptoms in the field as well as inability to detect BBTV in banana samples using PCR. Brooks, (1999) reported that the relatively low level of BBTV and its slow spread may indicate small populations or inactivity of the aphid vector, *Pentalonia nigronervosa* (Coquerel).

Two of the major problems that the farmers reported to affect banana cultivation were fire and theft. The farmers paint their banana with different colours and every farmer knows his colour for easy identification when stolen. Similar report was given by Mnyamwezi (2014) that farmers from western Kenya and Tanzania brand their names on banana fruit to prevent theft while some are opting to witch doctor services.

The identification of pests like grasshoppers and rodents feeding on banana agrees with the work of Viljoen, (2010) that damages in Banana plants were caused by pests such as the banana weevil and plant-parasitic nematodes. He also noted that the decline in yield/ha of *Musa* could be attributed to the increase in pathogen and pest

damage. More land was planted to banana with little effort made to increase yields in existing fields. Furthermore, the suckers primarily used as planting material were often infected with pathogens and pests. Other problems were water stress which result in death of some plants, overcrowding as the farmers don't prune the banana stands as well as insufficient manure.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 SUMMARY

1. Farmers are ignorant that the symptoms of the diseases observed are caused by viruses.

2. Of the four banana viruses BBrMV, CMV, BBTV and BSV tested with PCR/RT-PCR, BSV and CMV were detected in the study areas. Symptomatic banana samples were negative while some asymptomatic samples were positive for BSV using BSV Onne primer CMV, BSV and Potyvirus were detected in weed samples;

3. BSV was detected in some weeds for the first time in these areas.

4. None of the banana viruses were detected in both mealybugs and aphids.

6.2 CONCLUSION

1. The ignorance of the farmers about the disease resulted in improper management of the disease because they assume is caused by water stress, overcrowding and insufficient manure. During the sample collection, random sampling was initially employed but some farmers prevented us from collecting samples from their farms probably due to the security situation in the country. This resulted in the adoption of purposive sampling rather than random sampling.

2. The inability of BSV Onne primer to detect BSV in some symptomatic sample is an indication that the samples may be infected with another isolate of BSV since BSV Onne primer detects only BSV Onne isolate.

3. The detection of viruses in weeds indicates that the weeds analysed are good reservoir host of the viruses.

4. The inability of mealybug and aphid sample to amplify with PCR is probably because the samples (mealybug and aphid) have not acquired the virus or the samples (mealybug) contain another isolate of BSV.

6.3 RECOMMENDATIONS

1. Farmers should be enlightened about the disease.
2. As part of preventive strategy, farmers should obtain their suckers from healthy plants.
3. Weeding should be conducted regularly on banana farms or the farms should be sprayed with herbicide and insecticide.
4. Further research should be carried out to detect the other isolates of BSV in areas under study

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APPENDICES

Appendix 1: Questionnaire to Obtain Information on Banana Production Practices and Diseases from Banana Farmers

A. FARMER'S PERSONAL DATA

1. Name
2. Sex
3. location of the farm

B. FARM RECORDS

1. Banana or plantain?
2. What is the source of your Banana seed set?
3. How long have you been cultivating Banana?
4. How long does Banana take from planting to fruiting?
5. How do you maintain the Banana plants
6. What is the yield per Banana plant
7. Do you grow Banana for commercial, ornamental or consumption basis?
8. What is the market situation?
9. Varieties planted.
10. Name of the varieties.

C. What are the problems encountered in Banana cultivation? Please tick (√)

a. Planting materials

Source:

I. Generated from own old stock ☐

II. Or do you purchase it? ☐

III. Nongovernmental organization ☐

IV. Donated by government ☐

V. Local market ☐

b. Source of Water:

I. Rain fed ☐

II. Borehole ☐

III. Well ☐

IV. Tap water ☐

V. River/stream ☐

11. Have you noticed any disease on the plant?

12. Which part of plant is infected (stem/leaves/root)?

a. Pseudostem ☐

Types of symptoms:

b. Open leaves ☐

Types of symptoms:

c. Rolled leaves ☐

Types of symptoms:

d. Root ☐

Types of symptoms:

13. At what stage is the disease observed?

a. Seedling stage ☐

b. Maturity stage ☐

14. When does the disease occurs? Raining season ☐ Dry season ☐

15. What is the percentage of the diseased plant?

16. Is there any sample of the diseased plant?

    a. If yes, can we collect samples

    b. If no, when can we collect samples?

17. What are the effects on the crop?

    a. Is it on growth ☐

    b. Sweetness ☐

    c. Softness ☐

    d. Toughness ☐

    e. stunting ☐

    f. Others ☐ if other specify

18. What do you think contribute to the development of the disease?

    a. Rain ☐

    b. Wind ☐

    c. Insects ☐

    d. Humidity ☐

    e. Temperature ☐

    f. Herbicides ☐

    g. Fertilizer ☐

    h. Human ☐

    i. Others ☐

        I. If (g) yes, what type of fertilizer

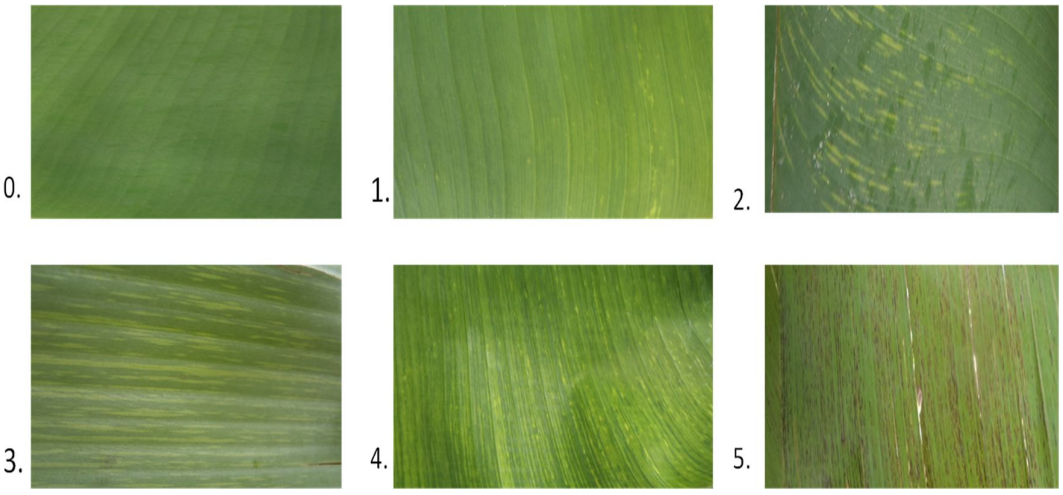
        II. At what stage do you apply fertilizer,

            Seedling ☐ Middle of Growth ☐ Maturity ☐

19. Is the disease symptom more on fertilized or unfertilized field?

20. What do you think cause the disease?

21. How do you manage the disease?



Appendix 2: Banana Leaves showing Symptoms Severity scoring scale. where 0 is no symptoms, 1 is localised flecks, 2 is scattered discontinuous streaks, 3 is continuous streaks covering moderate portion of lamina, 4 is continuous chlorotic streaks, and 5 is necrotic streaks (Karanja, 2010).

Appendix 3: ANOVA for Banana Samples

Source of variation	Sum of Squares	Df	Mean Square	F	Sig.
Farm	2.531	6	0.422	2.952*	0.017
PCR score	6.000	42	0.143		
Total	8.531	48			

There is significant difference in the PCR result between farms at ($p<0.05$) indicating that BSV is significant between farms.

Appedix 4: ANOVA for Symptoms Severity on Banana Leaves

Source of variation	Sum of Squares	df	Mean Square	F	Sig.
Farms	67.265	6	11.211	6.314**	0.000
Symptom severity	74.571	42	1.776		
Total	141.837	48			

There is significant difference in between the farms in terms of symptoms severity at ($p<0.05$) indicating that the severity of the disease depends on location.



Appendix 5: Fungal leaf blight.



Appendix 6: Grasshopper (*Zonocerus variegates* Linn) feeding on banana leaf



Appendix 7: Effect of rodents on banana fruit



(a)



(b)

Appendix 8: Effect of fire on banana

(a) Effect of fire on the banana plantation
(b) Effect of fire on banana fruit



(a)



(b)

Appendix 9: Prevention of theft by farmers

(a) Banana fruit painted with red ink
(b) Banana fruit painted with blue ink