OCCURENCE AND DISTRIBUTION OF PATHOGENIC VIRUSES AND FUNGI IN SWEET POTATO VARIETIES IN GWERU

By

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APPROVAL FORM

This is to certify that the dissertation entitled “Occurrence and distribution of pathogenic viruses and fungi in sweet potato varieties in Gweru.’’ is submitted in partial fulfilment of the requirements for Bachelor of Science Honours Degree in Applied Biological Sciences and Biotechnology at Midlands State University, is a record of the original research carried out by Samantha Chibanda under my supervision and no part of the dissertation has been submitted for any other degree or diploma.

The assistance and the help received during the course of this research have been duly acknowledged. Therefore, I recommend that it be accepted as fulfilling the dissertation requirements.

Name of supervisor …………………………………….

Signature …………………………………….

Chairperson signature

ABSTRACT

Viruses and fungi are the major threat to sweet potato production because they reduce yield by up to 90% thereby threatening food security. A study was carried out to identify pathogenic viruses and fungi affecting sweet potato and to determine their distribution across varieties and sites in Gweru. A total of 28 samples belonging to five different sweet potato varieties were collected from nine sites in Gweru. Plants were screened for viruses using two methods namely direct observations in the field and the use of *Ipomea indica* as a test plant. Attempts to use RT-PCR as a confirmatory test was unsuccessful because PCR did not work. Fungal culture on PDA and light microscopy were used for fungi identification. Samples belonging to different varieties were infected by either one of the following viruses or a complex of two of them - Sweet Potato Feathery Mottle Virus (SPFMV), Sweet Potato Chlorotic Stunt Virus (SPCSV), Sweet Potato Leaf Curl Virus (SPLCV) and Sweet Potato Mild Mottle Virus (SPMMV). SPFMV always occurred together with either SPCSV or SPLCV although SPLCV was sometimes found occurring on its own. SPMMV was always found in isolation. Occurrence of the viruses was in the ratio 38:21:20:5 for SPFMV+SPCSV, SPFMV+SPLCV, SPLCV and SPMMV respectively. SPFMV+SPCSV complex was the most widely distributed virus complex. MW variety was the least susceptible to devastating virus complexes such as SPFMV+SPCSV with an incidence of 12.5% but more susceptible to less harmful viruses such as SPLCV with an incidence of 50%. Widespread and spatial distribution patterns of viruses were possibly a result of continuous propagation of infected planting material over time or presence or absence of viral vectors in the different sites. Different fungal colonies that indicated presence of *Aspergillus spp, Alternaria alternata, Alternaria bataticola* and *Fusarium spp* were observed on the PDA. Prevalence of fungi was in the range of 5-58 counts for all varieties. Distribution of fungi in all nine sites sampled relies on dispersal mechanisms and moisture availability in the environment as in the case of *Fusarium spp*. GII and MW varieties were the least susceptible to fungal infections with prevalence of 25% and 13.33% respectively. The results obtained showed association in occurrence of viruses and fungi in sweet potato. This study has revealed that sweet potatoes in Gweru urban are infected by viruses and fungi, which can potentially lower yield by up to 90%. It is therefore imperative for the farmers to adopt control measures such as the use of virus free planting material and use of phytosanitation techniques to minimise post-harvest losses and eventually prevent it completely.

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DEDICATION

This research is dedicated to my loving parents Mr and Mrs Chibanda.

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Abbreviations

SPFMV	Sweet Potato Feathery Mottle Virus
SPCSV	Sweet Potato Chlorotic Stunt Virus
SPLCV	Sweet Potato Leaf Curl Virus
SPMMV	Sweet Potato Mild Mottle Virus
RNA	Ribonucleic acid
RT-PCR	Reverse transcription polymerase chain reaction
PDA	Potato dextrose agar
GII	German II
MW	Mozambican White
B	Brondal
C	Chingovha
W119	W119

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CHAPTER 1: INTRODUCTION

1.1 Background

Sweet potato (*Ipomea Batatas*), a member of the family *Convolvulaecea*, is one of the most cultivated root crops in Africa with countries such as Uganda and Rwanda producing more than two million tonnes per year and one million tonnes annually, respectively (Woolfe, 1992). It provides a diverse range of admirable traits such as its resistance to several confounding stresses from its environment but mostly consumed for its taste and nutritional value. Woolfe (1992) described sweet potato as having the lowest per capita income, amongst the major food crops. It holds great potential in benefiting poor household families and urban consumers at a large scale, since it produces more edible energy per hectare per day in comparison to other major food crops such as maize (Woolfe, 1992).

Zimbabwe is currently facing a food shortage, so in a bid to enhance food security sweet potato has become increasingly popular among subsistence farmers and some commercial farmers. FAO (2007) defined food security as a state in which all people at all times have physical, social and economic access to sufficient, safe and nutritious food to meet their dietary needs and food preferences for an active and healthy life. Sweet potato is regarded as a food security crop because of its high yields and low input requirements for production (Aritua and Gibson, 2002).

In a bid to diversify the usual African diet comprised of maize meal, the crop can be harvested and supplemented into the family's daily diet throughout the year (Bashasha *et al*., 1995). During the dry season and during seasons of scarcity sweet potato can be sliced into chips and dried. Dried sweet potato chips are a great source of carbohydrates and dietary fibre (Owori and Hagenimana, 2000). Sweet potato can also be processed to make flour which can be used for baking healthy gluten free products (Owori and Hagenimana, 2000). In Zimbabwe and several other African countries, the young leaves from the crop are eaten as vegetables (Owori and Hagenimana, 2000). The tuberous roots contain nutrients and minerals in varying proportions. The crop provides nutritionally significant quantities of ascorbic acid, riboflavin, iron, calcium and protein. In addition, the orange fleshed sweet potatoes such as Resisto and W119 are rich in β-carotene a precursor of vitamin A, which effectively fights certain types of cancer and other diseases. It is particularly valuable in meeting crucial dietary requirements of pregnant women and children .Traditionally the crop is grown mainly by

rural women near their homes to feed their families and to provide them with a source of income.

In order to reinforce our food security by increasing sweet potato production and productivity we need to understand the drawbacks that are associated with post-harvest losses of sweet potato. Challenges faced in sweet potato production include, diseases, pests, inadequate farmer access to improved cultivars, poor agronomic practices, and slow rate of technology adoption (Woolfe, 1992). Other constraints include poorly adapted cultivars, low yielding indigenous cultivars, poor marketing chain, low soil fertility and shortage of farm inputs (Woolfe, 1992).

A threat to sweet potato production is a threat to our food security and the greatest threat sweet potato varieties encounter is that of diseases. Before production can increase we need to understand diseases that affect sweet potato and in order to do this, the disease causing agents must be isolated and identified. Viruses and fungi which are responsible for significant yield losses are of particular interest in this research. Since sweet potato is propagated vegetatively by taking cuttings from previously sprouted tubers or directly from previously planted crops, build-up of virus and fungi infections in the different cultivars occurs over generations.

Viruses such as Sweet Potato Chlorotic Stunt Virus (SPCSV) and Sweet Potato Feathery Mottle Virus (SPFMV) for example exist as different strains which cause severe synergistic disease complexes which lead to a yield reduction which ranges from about 50-90% depending on severity of the infections (Chavi *et al.,* 1997). Land which would otherwise produce 30 tons per hectare in the absence of these two viruses ends up producing 6-8 tons per hectare. The extent of these losses poses a social livelihood as well as economic threat especially in a country such as Zimbabwe were food security has been compromised and this crop is the best alternative to sustain millions.

Fungal infections also pose a threat to sweet potato yield on a global scale (Ames *et al*, 1997). Fungal species associated with significant damage to the sweet potato are *Alternaria* species. These are responsible for diseases such as *Alternaria* leaf and stem blight disease. These cause a significant reduction in yield by reducing photosynthetic area and transport of

nutrients and other products to the tubers which are the storage roots and by causing vine death.

1.2 Problem Statement

Currently, there is only one documented research on identification of sweet potato viruses and none at all on sweet potato fungi in Gweru and Zimbabwe as a whole. Sweet potato viruses and fungi are responsible for synergistic disease complexes which result in significant yield reductions of up to 90%. Limited research on the agents responsible for devastation of a crop that can easily sustain the entire Zimbabwean population has sparked the immediate need of this research.

1.3 Justification

It is impossible to understand diseases responsible for yield reduction in sweet potato growing in Gweru without identifying the disease causing agents. Isolation , identification and characterisation of the disease causing pathogens is key to understanding diseases. Once we understand the disease it is easy to effectively integrate diseases management tools into farming practises. The fast deteriorating state of Zimbabwe's food security, with almost 4 million people being exposed to the risk of starvation in March 2017 calls for the immediate need to isolate and identify viruses and fungi responsible for significant post-harvest losses in sweet potato. Knowledge on the common viruses and fungi affecting sweet potato yield and how these pathogens are distributed in different varieties and areas will enlighten local Gweru farmers on what varieties are more susceptible to infection and which ones are more resistant, eventually leading to the mitigation of appropriate breeding programs of resistant varieties.

1.4 Objectives

The main objective was to isolate and identify viruses and fungi in different sweet potato varieties in Gweru.

The specific objectives were:

i. to determine susceptibility of different varieties of sweet potato to pathogenic viruses and fungi,
ii. to compare reliability of visual method and Indicator plant method with PCR as a standard, and

iii. to establish whether or not there are associations in occurrence of viruses and fungi species.

CHAPTER 2: LITERATURE REVIEW

2.1 Economic importance and geographical distribution of sweet potato

Sweet Potato (*Ipomea batatas*) is a dicotyledonous plant which belongs to the Convolvulaecea family. Amongst the genera and more than 1500 species belonging to this family, *Ipomea batatas* is the most important economically as a food crop (Wolfe,1992). It is the third most important root crop after potato and cassava and the seventh most important food crop after wheat, rice, maize, potato, barley and cassava in the world (Carey *et al.,* 1992). It is estimated that sweet potato cultivation in Uganda has extensively increased from 473,000 to 590,000 hectares in the past decade with an annual production of 2.2 million tonnes per year making it the third largest producer in the world after China and Nigeria (FAO, 2007). Its primary centres of origin are found in Central America and from there the secondary centres of genetic diversity are now in China, South East Asia and Africa (Carey *et al.,* 1992). Sweet potato is grown in the tropical and subtropical regions with low input but producing high yield under extremely marginal conditions

2.2 Sweet Potato Virus Characterisation

According to FAOSTAT data there are 8 million hectares of sweet potato being cultivated worldwide with approximately 103 million metric tons produced in 2012. Because of the increasing popularity of sweet potato and increased vegetative propagation, sweet potato viral diseases have become widespread causing major crop losses. Clark *et al* (2012) reported that more than 30 sweet potato viruses have been identified so far worldwide and the number increases each day as the virus detection methods are improved. Of the 30 viruses identified by 2012 only 23 were assigned formal taxonomic positions by the International Committee for Taxonomy Positions(ICTV) and even then only a few of those assigned taxonomic positions are of major economic importance. Sweet potato diseases are caused by both DNA and RNA viruses with RNA viruses being the most common causes. DNA viruses are single stranded and they fall in the family of *geminiviruses* under the genus *begomovirus* while RNA viruses fall under the genus *potyvirus*.

2.2.1 Sweet potato viruses common in Zimbabwe

In Zimbabwe sweet potato deterioration has been linked to viral infections. This is because farmers usually retain vine cuttings from previous growing season as seed for the next growing season. This perpetuates spread of infection and causes virus build up which

eventually leads to yield reduction (O Hair, 1991).Virus disease control would therefore require that one puts in mechanisms to quickly identify and detect viruses before clonal propagation. A virus elimination protocol such as that of meristem tip culture is also necessary to hamper further propagation of infected cuttings and improve yield (Chavi *et al.*, 1997).

Research carried out indicates that among viruses responsible for sweet potato deterioration , seven belong to the genus *potyvirus* and these are ,Sweet Potato Feathery Mottle Virus(SPFMV) ,Sweet Potato Mild Mottle Virus(SPMMV) ,Sweet Potato Latent Virus(SPLV) ,Sweet Potato Vein Mosaic Virus(SPVMV) ,Sweet Potato Virus 2(SPV2) ,Sweet Potato Symptomless Virus(SPSV) and Sweet Potato Yellow Dwarf Virus(SPYDV). In addition to these *potyviruses* are viruses such as Sweet Potato Leaf Curl Virus (SPLCV) which is a Begomovirus and Sweet Potato Chlorotic Stunt Virus (SPCSV) which is a *Crinivirus* (Karyeija *et al.*, 2000) .SPFMV is the most characterized sweet potato virus and it is known to infect the crop wherever it is grown (Brunt *et al.*, 1990) .Several strains have been identified which include East African (EA) strain, Ordinary (O) strain and Russet Crack. The virus is often identified as a component of synergistic complexes of whitefly transmitted *closteoviruses* and this interferes with identification of poorly characterized viruses (Cohen *et al.*, 1992). Despite having virus elimination protocols, virus indexing is not as easy because of the absence of rapid, sensitive and reliable diagnostic tools for the viral pathogens.

2.3 Symptomatology of viral infections and their associated diseases

Once a plant is infected, it exhibits different symptoms based on which virus or which combination of viruses has attacked the plant. Diseases associated with sweet potato viruses include, Internal Cork Disease which is caused by Sweet potato leaf speckling virus and Sweet potato vein mosaic virus .It is characterized by internal root necrosis, prominent in enlarged roots. Other symptoms associated with the disease include, Chlorotic leaf spotting, vein clearing, vein banding and purple ring spotting of foliage. Virus host range is limited to *Convolvulaceae* and the virus is transmitted by aphids (Clark, 1988).

Sweet potato Leaf spot disease is a non-persistent disease that is caused by Sweet potato leaf speckling virus transmitted by aphids. Clark, (1988) discovered that this disease has similar symptoms to internal cork which are Chlorotic leaf spotting ,vein clearing ,vein

banding and purple ring spotting of foliage. However there is no root necrosis in leaf spot unless leaf spot disease attacks a plant that is already infected with internal cork disease.

Mosaic disease is caused by a strain of tobacco mosaic virus known as Sweet Potato vein mosaic virus. In the USA the disease is associated with drying of the flesh of the Jersey sweet potato variety. The virus not only spreads by grafting from sweet potato to sweet potato ,there are incidences of spread by transfer from tobacco to sweet potato or vice versa. Other sweet potato varieties show great resistance to this virus (Clark *et al.*, 2002).

Sweet potato leaf speckling virus transmitted by the whitefly *Trialeurodes abutilonea*, causes Russet Crack disease. This disease is characterized by a russet type of discoloration and cracking of enlarged roots. Other symptoms include chlorotic spotting, followed by necrotic spotting of the foliage of certain varieties.

Clark (1988) describes, Feathery mottle complex disease as a complex of three diseases in one plant. Diseases that form this complex are Internal cork disease, leaf spot disease and a third disease which is caused by the Sweet potato yellow dwarf virus (SPYDV) transmitted by the whitefly *Bernisia tabaci* .On their own each disease has no effect on yield but together they result in a striking yield reduction that ranges from 10-80%.The disease is mainly characterized by dwarfing of plants.

Sweet Potato Virus Disease (SPVD) which is caused by Sweet potato feathery mottle virus and Sweet potato chlorotic stunt virus has the most devastating consequences in comparison to all the other diseases. SPFMV is transmitted by the whitefly while SPLCV is transmitted by aphids. SPVD is characterized by systematic vein clearing which is followed by remission (Clark and Moyer, 1998).

2.3.1 Economic importance of Sweet potato viral diseases

Most diseases associated with virus infections in sweet potato have no effect on yield but Sweet potato virus disease plays a significant role in reducing yield in sweet potato. This is because when viruses are singular, that is when they cause a single disease in plants they have minimal effect but when viruses combine the resultant diseases have devastating effects. The most devastating disease is Sweet potato virus disease (SPVD) which is caused by SPFMV which can exist as 3 different strains and SPCSV. SPVD can spread rapidly to uninfected plants, affecting 100% of plants in one season. Sweet potato virus disease results in a yield reduction of up to 90% (Chavi *et al.*, 1997).

Feathery Mottle Complex is also economically important because it leads to severe yield reduction which ranges from 10-80% .These are significant losses that can feed millions in Zimbabwe.

2.4 Virus detection techniques

Over time sweet potato viruses have been detected by observing symptom expression patterns in infected plants in the field and use of biological assays which include, observing sensitive indicator plants that have been inoculated by infected cell sap .Confirmatory biochemical assays and electron microscopy have also been used, as they have been presumed to be more precise and accurate in comparison to test plants. In as much as biological assays have been effective in detection of sweet potato viruses over the years they have limitations in that one has to be able to tell viruses apart in the event that there is co-infection as in the case of SPFMV, which is extremely difficult. Low concentration of sap, uneven distribution of the sap in the test plant and possible inhibitors of virus inoculation by plant tissue extracts also present a challenge but these can easily be controlled, if adjustments are made to the experiment beforehand (Chavi *et al.*, 1997).

According to Chavi *et al*, (1997) biochemical assays used, have been mainly based on immunodiagnostic techniques such as Elisa and filter binding. Elisa has been used on several occasions to identify SPFMV strains and sometimes Sweet potato mild mottle virus (SPMMV) and Sweet potato latent Virus (SPLV).Immunodiagnostic techniques are efficient in large scale epidemiological studies of sweet potato viruses despite the many limitations that surround them. Low concentration and uneven tissue to tissue distribution of sweet potato viruses reduce their reliability. High levels phenolic compounds, latex and other inhibitors in sweet potato tissue extracts also affect reliability and reproducibility of results.

Advances in molecular biology have led the revolution in rapid, sensitive and reliable detection of plant viruses based on synthetic nucleic acid probes or the in vitro amplification of specific nucleic acid (DNA) sequences by the polymerase chain reaction. This is a faster more reliable confirmatory method as compared to biochemical assays such as ELISA.

2.4.1 Host range test plants

Indexing based on grafts to susceptible test plants such as *Ipomea setosa* (Brazillian morning glory) is presumed to be very reliable in detection of some sweet potato viruses. Earlier studies indicated that this plant was a suitable host for all sweet potato infecting

viruses but recent studies have shown that viruses such as Tomato spotted wilt virus shows no visible symptoms on this host (Milleza *et al*., 2012). *Ipomea nil* is another host that shows visible symptoms in response to most sweet potato viruses. Mechanical inoculation with other host plants such as *I. indica, Nicotiana benthamiana, N.clevelandii, N. glutinosa, N. rustica*, and *N.tabacum* is recommended.

Since *I. Setosa* tests for a wide range of viruses especially those that affect African countries such as Zimbabwe, each *I. batatas* plant must be tested by graft inoculation using a minimum of 3 replicate indicator plants *of I. setosa.* Indicator plants must be maintained in a healthy, vigorous state, as symptoms associated with abiotic stresses, such as water and nutrient deficiencies, may mask and interfere with observations of disease symptoms. The indicator plants can be grown from seed or from young cuttings. Symptoms on *I. setosa* usually appear within 2-4 weeks. However, the severity of virus symptoms and length of time before they appear on the indicator plants depends upon the virus and the amount of virus inoculum present in the scion. The graft inoculation results will only be considered valid if, no symptoms are produced on the negative control (non-grafted) indicator plant and if the expected symptoms are produced on the indicator hosts with the positive control that is inoculated virus (Chavi *et al*., 1997).

2.4.2 Enzyme -Linked Immunosorbent Assay (ELISA)

ELISA refers to a plate-based assay technique designed for detecting and quantifying substances such as peptides, proteins, antibodies and hormones. In an ELISA, an antigen must be immobilized to a solid surface and then complexed with an antibody that is linked to an enzyme. Detection is accomplished by assessing the conjugated enzyme activity via incubation with a substrate to produce a measureable product (Gibson *et al*., 1998). The most crucial element of the detection strategy is a highly specific antibody-antigen interaction. ELISA in sweet potato virus detection employs the use of three different methods. These three methods are, Double antibody sandwich ELISA (DAS-ELISA), dot ELISA and direct ELISA.

2.4.3 Reliability of ELISA techniques in viral identification

Ashoub *et al*. (2008) concluded that, when the standard DAS-ELISA (Clark and Adams, 1977) technique was used to distinguish between healthy and SPFMV-infected

sweet potato plants, results indicated that differences between values of healthy samples readings (0.411) and infected samples readings (0.473) were not sufficient to distinguish between healthy and infected plants.

However, modifications made to direct ELISA, by applying the samples directly to the plates , followed by washing steps improved the efficiency of the method and resulted in clear cut distinguishing between healthy and infected samples, with infected sample readings (1.399) being approximately 3 times higher than the healthy sample readings (0.468) when sodium carbonate-bicarbonate buffer pH 9.6 was used as sample extraction buffer and and infected samples readings (1.015) were about 2.5 times as high as the healthy sample readings (0.410) when PBS pH 7.4 was used as sample extraction buffer (Ashoub *et al* ., 2008).

Results obtained from the dot-ELISA also indicated a desirable level of efficiency to distinguish between healthy and SPFMV infected sweet potato plants .Since the viral protein is attached directly to the supporting material during the washing and blocking steps, removal of inhibitory factors present in the sweet potato extracts is improved, resulting in better specific binding of the virus to the antibodies against SPFMV. In this study, nitrocellulose membranes were replaced by positively charged membranes because they are relatively easy to handle.

Ashoub *et al.* (2008) concluded that DAS-ELISA is not adequate for the detection of SPFMV since almost identical ELISA values were obtained for healthy and infected plants. In contrast, reliable results were obtained in detection of SPFMV by either direct or dot ELISA with quantitatively and qualitatively clearly distinguishable values for infected and non-infected material. For high specificity RT-PCR was highly recommended in the conclusions. PCR utilises specific primers which are based on the conserved regions from all viral strains obtained from the NCBI database. The advantage of using these specific primers is that, they are universal primers that can detect any SPFMV-infected sweet potato material regardless of the viral isolate. The resulting amplification product was 300 nucleotides when he carried it out and present only in infected plants. There were no additional dominant bands detected in either the healthy or infected plant materials.

2.4.4 Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)

The polymerase chain reaction is a technique used to exponentially amplify target DNA sequences. It makes use of the template DNA, the forward and reverse primers and the master mix which includes *Taq* DNA polymerase, dNTP's, $MgCl_2$ and reaction buffer.

RT-PCR a variant of PCR involves the use of reverse transcriptase enzyme to synthesize a complementary DNA strand from RNA in an incubator or thermal cycler. RT-PCR usually requires the use of rapid kits which make RNA extraction and isolation easier. RT-PCR first clones expressed genes by converting RNA to cDNA by use of reverse transcriptase before the cDNA is then amplified (Mackay, 2007). cDNA strand provides the sequences from which the primers can extend during the five standard cycles which are Initial denaturation(94 °C), denaturation(94°C), annealing (58 °C), extension (72 °C) and final extension (72 °C) of the Polymerase chain reaction in the thermal cycler.

RT-PCR can occur in a one- step procedure or a two -step procedure. According to Radonić *et al*. (2004) in a one-step RT-PCR procedure ,the entire reaction from cDNA synthesis to PCR amplification occurs in a single PCR tube wile in the two-step procedure, cDNA synthesis in the reverse transcriptase reaction is separated from PCR amplification(occur in two different PCR tubes). The One step procedure minimises experimental variation by containing enzymatic reactions in a single environment although there are very high chances that RNA will degrade especially when repeated assays from the same sample is required. The two step procedure is highly recommended in RNA extraction and amplification because after reverse transcriptase cDNA is stable at the first level and you can check for its integrity by running gel electrophoresis and it can easily be stored at -20 °C. One can check for integrity of the nucleic acid by running gel electrophoresis. Susceptibility to contamination is very high because the two step procedure requires a lot of handling.

2.4.5 Primer design

Primers are short nucleotide sequences that act as the starting points for DNA synthesis in the polymerase chain reaction. The DNA polymerase extends from the 3' end of the primer and copies the opposite strand.

According to Ausubel *et al*. (2000) it is generally accepted that the optimal length of PCR primers is 18-22 base pairs. This length is long enough for adequate specificity and short enough for primers to bind easily to the template at the annealing temperature. The melting temperature (Tm) of the primer which is the temperature at which the DNA

double helix unwinds to become single stranded should range from 52 ^{o}C -58 ^{o}C to produce the best results. Primers with melting temperatures above 65°C are prone to secondary annealing. Annealing temperature is determined by the melting temperature which is the estimate of the DNA-DNA hybrid stability. If annealing temperature is too low, it may lead to production of non-specific PCR products caused by base pair mismatches and if it is too high it will result in insufficient primer template hybridisation leading to low PCR product (Ausubel *et al.*, 2000). It is important to ensure that both primers have approximately the same annealing temperature (Tm) so that they anneal at just about the same time. The acceptable difference between the two is 2 °C.

GC content which is the amount of bases G and C in the primer as a percentage, should range from 40-60%. Presence of G's and C's at the 3'end promotes specific binding due to the stronger bonds formed between G and C bases (Ausubel *et al*., 2000) .Primers should also avoid formation of primer secondary structures. Primer secondary structures produced by intermolecular or intramolecular interactions can lead to poor or no yield of the product. They adversely affect primer template annealing and thus the amplification. They greatly reduce the availability of primers to the reaction. The secondary structures include; a hairpin formed by intramolecular interactions within the primer, Self Dimer which is formed when there are intermolecular interactions between two same sense primers. In this case the primer is homologous to itself. When primers form intermolecular dimers much more readily than hybridizing to target DNA, they reduce the product yield .The last secondary structure is a Cross dimer which is formed by intermolecular interactions between sense and antisense primers, where they are homologous (Ausubel *et al.*, 2000)

According to Ausubel *et al*, (2000) repeats must be avoided in primer design. A repeat is a di-nucleotide occurring many times consecutively and should be avoided because they can misprime. The maximum number of di-nucleotide repeats acceptable in an oligo is 4 di-nucleotides. It is important to design primers in the regions of the template that do not form stable secondary structures during the polymerase chain reaction. This is because if primers are designed on stable secondary structures, even above annealing temperatures the primers area unable to bind to the template and the PCR yield is significantly affected (Ausubel *et al.,* 2000). Cross Homology should also be avoided in primers. This means that one should make sure that primers designed for a specific gene of interest should not amplify other genes in the sample. BLASTing the primer to test for specificity in the NCBI database for example will ensure that there is no cross homology by identifying

regions of significant cross homology and avoiding them during primer search (Ausubel *et al.,* 2000).

2.4.6 Gel Electrophoresis

Gel Electrophoresis is a technique used to separate fragments of molecules such as DNA, RNA and proteins based on their charge and size. Distance travelled is inversely proportional to their size, so larger molecules will travel a shorter distance whilst smaller ones, travel a greater distance. An electrical field is applied with one being negatively charged and the other positively charged. Since RNA and DNA are negatively charged they will move towards the positively charged end of the gel (Buckingham and Flaws, 2007). Different concentrations of agarose resolves different sizes of DNA fragments with higher concentrations facilitating separation of small pieces of DNA while lower concentrations allow resolution of larger DNA fragments. Electrophoresis buffer such as Tris-acetate-EDTA (TAE) and Tris-borate-EDTA (TBE) are used in gel electrophoresis and DNA fragments migrate at different rates in the two buffers because they have different ionic strength (Buckingham and Flaws, 2007).A fluorescent dye such as Ethidium bromide which fluoresces the DNA is added to the agarose gel before it solidifies. .A molecular weight marker can be used to compare samples when the presence of a pathogen is being investigated. To observe bands the gel is viewed under UV light in a UV trans illuminator.

2.4.7 Identification of sweet potato viruses using RT-PCR

Abad and Moyer, (1992) developed in vitro transcribed RNA (cRNA) probes also known as ripobrobes. These probes detected some of the currently recognized SPFMV strains using cDNA containing the 3´ terminal region of the capsid protein cistron, which exhibited a high degree of homology for the potyvirus group. Riboprobes showed greater sensitivity than did immunodiagnostic filter binding, because the riboprobe system overcame interference with host factors that compromise the reliability of immunodiagnostic assays in SPFMV detection.

Colinet and Kummert (1993) developed a PCR protocol that distinctly identified sweet potato potyviruses in infected sweet potato in China. Using degenerate primers derived from conserved sequence motifs in the genomes of potyviruses designed to amplify the variable 5´ terminal region of the capsid protein cistron, three different potyviruses which are a Chinese

isolate of SPFMV, SPLV, and a potyvirus closely related to SPFMV were identified by the amplification of the RNA obtained from the sweet potato clones originating from China

In another study conducted in Korea by Kwak *et al.* (2014), Single and Multiplex RT-PCR assays were used to successfully detect eight major sweet potato viruses affecting sweet potato in Korea. Experiment was originally set up for detection of 17 viruses although only eight were successfully identified which might be an indication that sweet potato in Korea is only susceptible to these eight viruses. *Sweet potato feathery mottle virus* (SPFMV) and *sweet potato virus* C (SPVC) were most commonly detected, infecting approximately 87% and 85% of samples, respectively. Furthermore, *Sweet potato symptomless virus* 1 (SPSMV-1), *Sweet potato virus* G (SPVG), *Sweet potato leaf curl virus* (SPLCV), *Sweet potato virus* 2 (SPV2), *Sweet potato Chlorotic fleck virus* (SPCFV) and *Sweet potato latent virus* (SPLV) were detected in 67%, 58%, 47%, 41%, 31%, and 20% of samples, respectively. This study was the first official documented study of the occurrence of four viruses (SPVC, SPV2, SPCFV, and SPSMV-1) in Korea.

Chavi *et al*. (1997) successfully identified strain of SPFMV using RT–PCR. The data obtained, shows that the degenerate primers designed from conserved regions of the potyvirus genome for the detection of sweet potato viruses, did detect the virus isolated and purified from a Zimbabwean sweet potato clone, which showed close serological relationships with a well-characterized strain of SPFMV, the russet crack strain. Further characterization of the vein clearing inducing agent was also attempted by reverse transcription-polymerase chain reaction, by amplification of total RNA with degenerate primers for potyviruses and an oligo dT primer and PCR products of correct size were obtained. The nucleotide sequence was determined and the amino acid of the poly-protein deduced. Comparison with other strains of SPFMV showed strong similarity except for an insertion of 22 amino acids at the N-terminus of the coat protein. The coat protein size of 335 amino acids is the biggest SPFMV so far determined in Zimbabwe (Chavi *et al*., 1997).

2.5 RT-PCR trouble shooting

It is very important to ensure that all the processes of the PCR, RT-PCR and qPCR are fully understood so that data obtained is reliable and any problems encountered can be fully addressed. In the event that PCR is said to be unsuccessful, any possible source of error despite how insignificant it may seem, should be explored independently until the

source of the problem is identified. Mistakes made in handling the master mix can be a source of failure in amplification during PCR. It is important to note that different master mix products are particularly sensitive to buffer compositions and primer concentration combinations especially if one want to switch master mixes for a particular reaction. Before one can make a radical decision on switching master mixes one should verify its optimal conditions (Fenby *et al*., 1998).

If oligos are poorly designed, have an incorrect sequence or if they are run under sub optimal conditions, they will yield no product and data if any at all is obtained. A poor design can also lead to a misrepresentation of the genuine biology which is being studied. When an assay fails yet there are no mistakes in the design or operating procedures, assay optimisation by increasing primer concentrations is a good start in finding out whether optimisation will improve assay (Fenby *et al*., 1998). Instrument failure is also very common in running a Polymerase chain reaction. Use of control samples is invaluable when carrying out PCR. The optimised assay will act as the control, which helps identify whether or not the instrument is working properly (Fenby *et al*., 1998).

Checking for the quality of the template is also very important in troubleshooting. Template quality encompasses, template quantity, integrity and the presence of inhibitors .Best quality template should be used. RNA should be handled carefully in experiments as it easily degrades and it should be matched to the most appropriate Reverse Transcriptase protocol.

Fenby *et al*. (1998) describes a test that was carried out on a serial dilution using an artificial oligo, a standard primer and a probe assay. Assay had originally been developed in a different lab, using a different instrument and with a different operator and the dilution effect was not expected as reflected by the standard curve obtained. Conditions were re-optimised in a different lab and the same experiment was carried out using a different instrument and different operator but the dilution effect was even more pronounced. From the examination it, the instruments were working and both the operators were very experienced so of the three things suspected to be the source of variability the third option which was suggested as some subtle variation in experimental procedure was examined. The tubes used in the dilution series were examined and a number of the tubes were used as tests. On switching to 1.5 ml eppendorf tubes the predicted standard curve was generated. The conclusion made was that low retention plastic ware is designed for PCR and protocol should be observed because a

slight deviation can affect our results as assays are sensitive to subtle variation from the protocol.

2.6 Sweet potato fungi characterisation and taxonomy

Fungi threaten to wipe out sweet potato yields, if not identified and carefully managed. Fungal species associated with sweet potato diseases are *Alternaria* species which are responsible for *Alternaria* leaf and stem blight diseases. *Alternaria* species belong to the family *Dematiacea*, order *Monilales*, and class *Deuteromycetes* (David, 1991). This genus is identified by ellipsoidal conidia with many transverse and longitudinal septa and a beak like structure on the distal end (David, 1991).Exact shapes and sizes vary with species. Species associated with diseases that cause great damage to sweet potato are, *Alternaria spp Alternaria alternata, Alternaria capsici-annui* and other associated fungi include *,Alternaria solani, Alternaria tenuisa, Alternaria tax spp(iv), and Alternaria bataticola* (Lenne ,1991). *Alternaria alternata* has a microscopic morphology that is branched with acropetal chains of multicellular conidia which are produced sympodially from simple, sometimes branched, shorter elongate conidiophores. David (1991) referred to the conidia of *A.alternata* as obclative, obpyriform, and ovoid or ellipsoidal often with a short conical or cylindrical beak and have a pale brown, smooth walled verrucose. Its conidia are not singular but rather formed as short chains formed from conidiophores. Just like *Alternaria solani*, conidia are multiple, septate and muriform. It is only the profuse aerial mycelium that is lighter in colour in comparison to that of *A. solani*.

2.7 Sweet potato fungal diseases distribution and incidence

Alternaria fungi species infections in sweet potato, result in *Alternaria* leaf petiole stem blight disease, *alternariasis* and *anthcnose* with leaf petiole and stem blight disease being the most common and most severe. These two sweet potato diseases have been reported in a number of sweet potato producing countries which include ,Rwanda with *Alternaria solani* being the cause of diseases , Zimbabwe with *Alternaria spp* being the causative agent (Whiteside ,1966) ,Ethiopia with *Alternaria tax spp(iv)* (Van Bruggen ,1984) and in Burundi with *Alternaria solani* (Simbashizweko and Perredox ,1988) being the fungi responsible for disease occurrence.

Disease incidence and severity of infection was higher than 50% in highland areas. Severity of the disease is serious at higher altitudes in infertile acidic soils in Rwanda although in Ethiopia it is the sweet potato that is located at medium altitude regions that is

affected. Leaf damage which reduces photosynthetic area is often the initial effect of *Alternaria* leaf spot. Severe attacks are responsible for vine death which results in 100% crop failure while milder attacks cause defoliation. Premature defoliation results in stunted plant growth and development which influences production negatively. Stem blight is manifested in wet areas and stem necrosis is manifested in drier areas. If the main stem is affected when the plant is young the plant dies. With stem blight, attacks in the later stages results in no yield loss. *Alternaria* fungi species spread though infected plant material and by means of dispersal methods such as wind and water (Ames *et al.*, 1997).

2.8 Symptomatology and identification on sight.

Alternaria leaf spot is characterised by leaf spots ranging from faint discoloured spots to marked necrotic annular lesions (Skoglund and Smit, 1994).Stem blight and *Alternaria* petiole appear as small grey to black oval lesions which exhibit lighter centres on the stem and petiole. Lesion expansion on the leaves is limited to the mid rib and veins causing anthracnose like symptoms. Coalescence of the lesions results in blighting of the leaf and where they do not coalesce the area adjacent to the veins becomes yellow and the leaves easily detach from the plant. Under humid conditions lesions enlarge as black areas resulting in stem and petiole girdling. Brown lesions that appear like concentric rings occur on leaves with higher incidence occurring on older leaves (Skoglund and Smit, 1994). When the plant is still young, the plant dies and if it's old the leaves above the affected part die (Van Brussen, 1994). Black leaf debris is found on the ground where affected vines are growing. The bases and middle sections are more affected than the vine terminals.

2.9 Cultural studies of Alternaria species

Alternaria species grow and sporulate in various media but maximum growth and sporulation occurs on Potato Dextrose Agar (PDA) (Van Brugen, 1984) .Light also influences growth and sporulation of *Alternaria* species. For some species, optimal conditions are in continuous light while for some optimal conditions are under continuous darkness or alternation of light and darkness for specified hours. Temperatures suitable for optimal growth are within the 15 °C -30°C range.

2.10 Screening for resistant varieties

To determine resistance of sweet potato varieties to the different diseases caused by fungi, an assessment of disease incidence and severity in plant populations can be carried

out. Other screening methods include, study of disease using areas under disease progress curves .Van Brugen, (1984) reported the screening of sweet potato for resistance to *Alternaria tax sp (iv)* and *Alternaria solani* in Ethiopia and Rwanda respectively. As reported by Van Bruggen (1984), of the 13 varieties he evaluated, there were clear differences in susceptibility to the two fungi species among varieties. Varieties with red tubers (Koka 25 and Koka 12) seemed to be more resistant than those with white tubers (Koka 9'A' and Abotso).The chi square test used for analysis showed the differences for the two viruses were not statistically significant despite the fact that none of the varieties tested were immune.

CHAPTER 3: MATERIALS AND METHODS

3.1 Study site

Sweet potato leaf samples exhibiting disease-like symptoms were collected from Gweru urban areas; Mkoba 11, Mkoba 13, Mkoba 14, Mkoba 6, Mkoba 10, Nashville, Kopje, Senga and Ivene. Further analyses on the samples were carried out at the MSU Biotechnology Laboratories. Study sites as shown in Fig 3.1(a) and 3.1(b) were chosen based on sweet potato subsistence farming in the areas.

Fig 3.1(a) Map of Zimbabwe showing study area chosen for the research

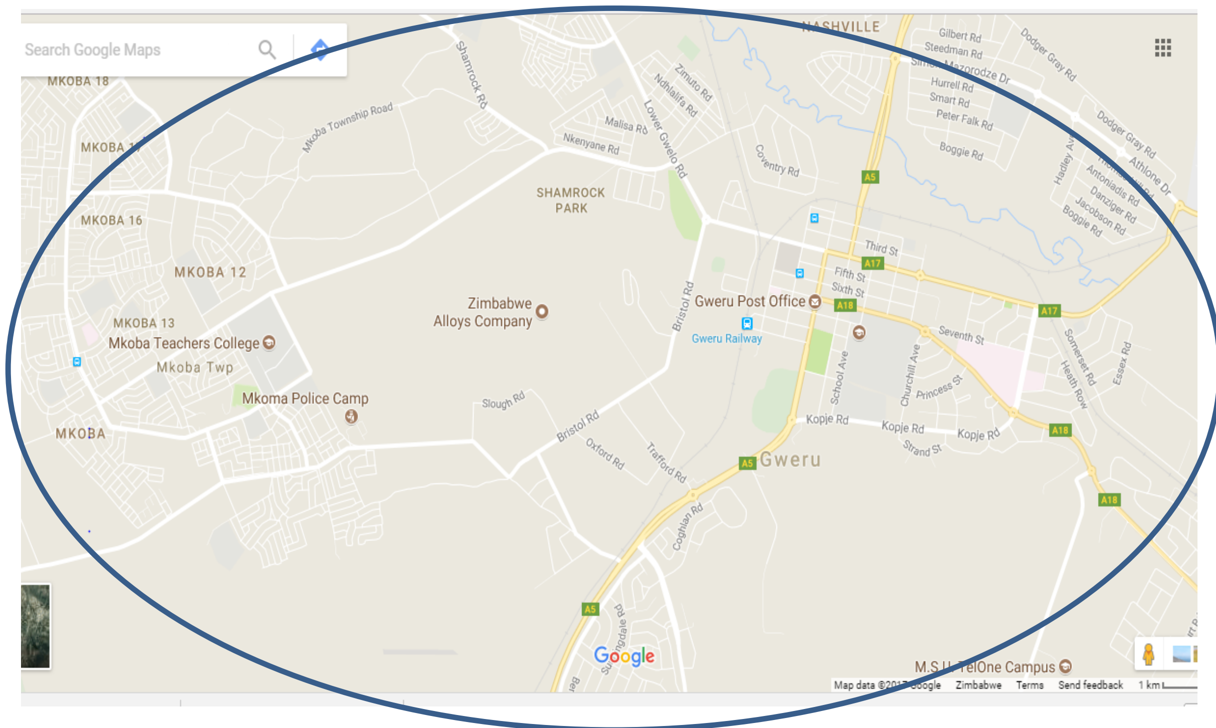


Fig 3.1(b) Map of Gweru showing specific study sites chosen for this research

3.2 Study design

The experimental design used in the study was a Completely Randomised Design. Simple random sampling was used in collection of different *Ipomea batatas* leaves. From each sampling site, 3 samples were collected for each sweet potato variety. A total of 5 different sweet potato varieties (Chingovha, Brondal, German II, Mozambican White and W119) were sampled from each different site.

3.3 *Ipomea indica* planting and mechanical inoculation for virus testing

Prior to sample collection and mechanical inoculation, vine cuttings of *Ipomea India,* a virus indicator plant, were collected from Golden Stairs and replanted in pots at Midlands State University under greenhouse conditions. The *Ipomea India* vine cuttings were planted in pine bark obtained from the Midlands State University Agriculture Department. The pot plants were watered at two-day intervals over a period of three weeks. At four weeks sticks of uniform size of 60 cm were put in the pots to allow the test plants to climb. A total of 24 pot plants were planted with *Ipomea batatas*. Using a pestle and mortar three leaf samples of each sweet potato variety from every study site were ground until cell sap could be observed. Sample was homogenised in mortar containing 13 ml of 0.1 M potassium phosphate buffer pH 7.2 supplemented with 0.01 M sodium sulphite. Sample sap was then rubbed onto the indicator plant leaves which were previously brushed lightly with carbonidium (sandpaper). Inoculated indicator plants were taken back to the greenhouse after rubbing leaves with

running tap water. Plants were observed over a period of 4 weeks and they were constantly watered at 2 day intervals.

3.4 Polymerase chain reaction for virus testing

3.4.1 Primer design

Primers for the detection of sweet potato viruses were designed based on the nucleotide sequences of different sweet potato viruses registered in GenBank of the National Centre of Biotechnology Information. Table 3.1 shows the primers designed and used in the detection of Sweet potato viruses.

TABLE 3.1 Primers designed for virus identification

Virus	Primer	Sequence	Length(bases)
Sweet Potato Feathery Mottle	SPFMV (F)	TACACACTGCTAAAACTAGG	20
Virus(SPFMV)	SPFMV (R)	AGTTCATCATAACCCCATGA	20
Sweet Potato Leaf Curl Virus	SPLCV (F)	TCTGCCGTCGATCTGGAACTC	21
(SPLCV)	SPLCV(R)	GTGCCCGCCTTTGGTGGAC	19
Sweet Potato Chlorotic Stunt	SPCSV (F)	GGGAAGAMGAGAYATGGAGTTAA	23
Virus (SPCSV)	SPCSV (R)	CCTTGTTACAAAGAGCGTTCCT	22
Sweet Potato Symptomless	SPSMV(F)	ACCGTGTATTTGATGACGATGTAC	24
Virus(SPSMV)	SPSMV(R)	GGGAAGTTCTGGTAGAACGTATC	23
Sweet Potato	SPV2(F)	ATGTGTTGAACCATCAGCTGAA	22
Virus (SPV2)	SPV2(R)	GTAACTTGCCTTGGGCTACG	20

3.4.2 Identification of viral genetic material by Reverse Transcriptase -Polymerase Chain Reaction amplification

Cells were lysed by exposing the leaves to liquid nitrogen and crushing using pestle and mortar. Leaf samples weighing 2 g each were solubilised in 20 ml of extraction buffer (10 µM EDTA, 1M NaCl, 1M Glycine,10% Sodium dodecyl sulpfate and 50mg/ml bentonite). RNA was then extracted from the solubilised leaf samples using the Viral gene-spin™ viral DNA/RNA extraction kit from ZYMO RESEARCH according to the manufacturer's instructions.

RT-PCR Assays were carried out in a two-step procedure which involved the use of AMV reverse transcriptase for reverse transcription and Go-taq polymerase for PCR. RT-PCR assays were performed using two-step RT-PCR. For the first step, reverse transcription reactions were carried out in a final volume of 5 µl reaction obtained by combining 0.5 µl total RNA, 0.5 µl of a mixture of 32 µM reverse primer, 1 µl 5 × RT buffer, 0.5 µl 2.5 µM dNTP, 8 U RNase inhibitor, 4µl of 0.5 U AMV reverse transcriptase, and sufficient distilled water to bring the total to 5 µl. The thermal cycler was set at 45°C and RT reactions were terminated by heating at 95 °C for 5 min.

For the second step of the reaction , a volume of 20 µl of a solution comprising 0.5 µl of 32 µM forward primer, 5 µl 5 × PCR buffer, 2.5 µl of 25 mM $MgCl_2$, 1 U Go-Taq DNA polymerase and distilled water was added to the tube containing RT products. PCR was performed in a thermal cycler with the following conditions: Pre Denaturing for 5 min at 94 °C; Denaturing at 94 °C for 30 s for 35 cycles , Annealing 55 °C for 30 seconds, Extension at 72°C for 1 minute, and Final Extension at 72 °C for 5 min. PCR products were analyzed by electrophoresis on a 1.5% agarose gel at 100 V for 90 min, stained with EZ- Vision® Bluelight DNA dye, and DNA bands were visualized using a UV Tran illuminator.

3.5 Fungi testing

3.5.1 Media Preparation

Potato dextrose agar (PDA) media was prepared according to the manufacturer's instructions as indicated on the container. The media was then autoclaved at 121 °C for 15 minutes. After autoclaving the medium was transferred to a sterile laminar flow cabinet. The medium was left to cool for about 30 minutes before pouring into sterile petri plates. Media was left to solidify in the laminar flow cabinet. A total of 81 plates was prepared and left overnight to check for effectiveness of aseptic techniques.

3.5.2 Isolation of fungus

A total of three leaves for each variety was taken from each of the samples (15 leaves each) collected from each study site. Fungi isolation from leaf samples was carried out according to the protocol by Suleiman and Falaiye (2013). Samples were labelled and put into the oven for drying. They were oven dried for 20 h at 35 °C and temperature was carefully monitored, so as to avoid killing extracellular and intracellular fungi. After drying, leaves were ground into a fine powder using a pestle and mortar. A volume of 10 ml of distilled water was added to each powdered sample to form a liquid sample. Liquid samples were transferred to the laminar flow cabinet for inoculation. A volume of 3 ml of the sample was added to the Petri dish with media. This was replicated in two other petri dishes to make a total of three replicates for each sample. Spread plating was used to inoculate fungi samples onto the media. A control was set up identical to the experiments except for the inoculation. Petri dishes were sealed with parafilm to avoid contamination of media by other external microorganisms in the environment. Cultures were taken from the laminar flow cabinet and transferred to the incubator were they were incubated at 30 °C for four days. Growth of fungi was monitored daily. There was no microorganism growth observed in our control after the four days so aseptic techniques employed in culturing were effective.

3.5.3 Identification of fungi under the light microscope

Fungal cultures were taken out of the incubator and transferred to the workbench. A drop of methylene blue stain was added to the sterile slides using a dropper .A sterile inoculating loop was then used to transfer the cultured sample onto the stained slide. Before the stain dried out, it was covered with a sterile coverslip before it was observed under the light microscope.

3.6 Data analyses

Data were analysed using Microsoft Excel bar graphs for incidence of viruses and fungi, susceptibility of different varieties to viruses and fungi and relationship in occurrence of viruses.

CHAPTER 4: RESULTS

4.1 Occurrence and incidence of viruses

Viruses were successfully identified by the visual method and the test plant method. RT-PCR did not work, therefore all the results reported here are based on the visual and test plant methods only. A total of four viruses were observed in the 28 sweet potato samples collected from the nine sites. The four viruses comprised sweet potato feathery mottle virus (SPFMV), sweet potato chlorotic stunt virus (SPCSV), sweet potato leaf curl virus (SPLCV) and sweet potato mild mottle virus (SPMMV). SPFMV always occurred together with either SPCSV or SPLCV.

4.1.1 Identification of viruses by viewing samples in the field

Viruses were identified by observing the symptoms exhibited on leaves of samples collected. The viruses responsible for the symptoms observed on the leaf samples collected from the nine different sites were, SPFMV, SPCSV, SPLCV and SPMMV. SPFMV was always identified in synergy with another virus which was either SPCSV or SPLCV. From the 28 samples collected incidence of the viruses, SPFMV+SPCSV, SPFMV+SPLCV, SPLCV and SPMMV was in the ratio 13:7:7:1 respectively.

SPFMV+SPCSV was the most prevalent and infected samples exhibited narrow leaves that were yellowing and had deformed edges. Fig 4.1 shows an example of a GII sample infected by SPFMV +SPCSV from Site 9. There was widespread distribution of the synergistic viral complex in eight sites out the total nine sites. Varieties infected were ;Brondal (B) from Site 1 ;W119 from site 2 ;Chingovha(C) from Site 3 ;B ,Mozambican White (MW) and W119 from Site 4 ;B from Site 5 ;B and German II from Site 6 ;B and GII from Site 7 ,no viral incidence in Site8 and GII ,B and C in Site 9.

Fig 4.1: GII sample from site 9 infected by SPFMV+SPCSV

The seven samples that were infected with SPFMV + SPLCV as shown were identified by the yellow netting they exhibited and the rolling of leaves. Fig 2 shows an example of a GII sample infected by SPFMV +SPLCV from Site 4 .There was spatial distribution of the virus in four sites. Infected samples were varieties , C from Site 1 ;MW from Site 2 ;MW AND B from Site 3 and 3 C samples from Site 8 .There was no incidence of SPFMV +SPLCV in varieties from Sites 4,5,6 ,7 and 9.

Fig 4.2: GII sample from site 4 infected by SPFMV+ SPLCV

Prevalence of SPLCV (7counts) was the same as SPFMV+SPLCV prevalence. Infection by SPLCV was characterised by curling of the leaves. Fig 4.3 shows an example of a B sample infected by SPLCV from Site 3 .There was spatial distribution of SPLCV in six sites. Varieties infected were MW from Site 1; B from Site 2 ; B from Site 4 ;MW and C from Site 6 ; MW from Site 7 and MW from Site 9 while was no viral incidence in varieties from Sites 3 ,5 and 8

Fig 4.3: B sample from site 3 infected by SPLCV

Prevalence of SPPMV (1count) was very low in comparison to other viruses. Distribution of the virus was only limited to site 4.The SPMMV infected MW sample collected from site 4 showed signs of mottling and stunted growth. Fig 4.4 shows the MW sample infected by SPMMV from Site 4.

Fig 4.4: MW sample from site 4 infected by SPMMV

4.1.2 Identification of the Viruses using *Ipomea indica* test plant

The highest viral occurrence in *Ipomea indica* was recorded for SPFMV+SPCSV which had 32 counts as shown in Fig 4.10. Test plants infected by SPFMV+SPCSV showed signs of stunted growth, spotting and yellowing at the edges (Fig 4.5). There was widespread distribution of the synergistic viral complex in samples collected from eight sites out the total nine sites.

Fig: 4.5 *I. indica* infected by SPFMV+SPCSV, B sample from Site 6

SPFMV+SPLCV had the second highest occurrence of 21 counts (Fig 4.10). There was spatial distribution of the virus complex in four sites. Infected test plants showed signs of leaf curling with the leaf's edges curling towards the inner surface of the leaf ,yellowing of leaves and slight spotting(Fig 4.6).

Fig 4.6: *I. indica* infected by SPFMV +SPLCV, MW from site 4

SPLCV followed with a 20 count (Fig4.10) occurrence among the test plants. There was spatial distribution of SPLCV in six sites. Infection by SPLCV was characterised by leaf edges curling towards the inside of the leaf. Plants showed no other viral infection signs (Fig4.7).

Fig 4.7: *I. indica* infected by SPLCV, MW sample from site 2

SPMMV had the lowest occurrence having a count of 5 (Fig 4.10). Distribution of SPMMV was restricted to sites 4 and 6. Fig 4.8 shows a test plant that shows signs of mild mottling and slight spotting 4 weeks after inoculation with sap from an MW sample from Site 4.

Fig 4.8:*I.indica* infected by SPMMV, MW sample from site 4

Four weeks post inoculation the control exhibited no signs of infection (Fig 4.9)

Fig 4.9:*Ipomea indica* uninfected control

Viral incidence of SPFMV+SPCSV, SPFMV+SPLCV, SPLCV and SPMMV was in the ratio 32:21:20:5 respectively. There was widespread distribution of SPFMV+SPCSV, spatial distribution of SPFMV+SPLCV and SPLCV and SPPMV restricted to infection with samples from two sites as shown in Fig 4.10.

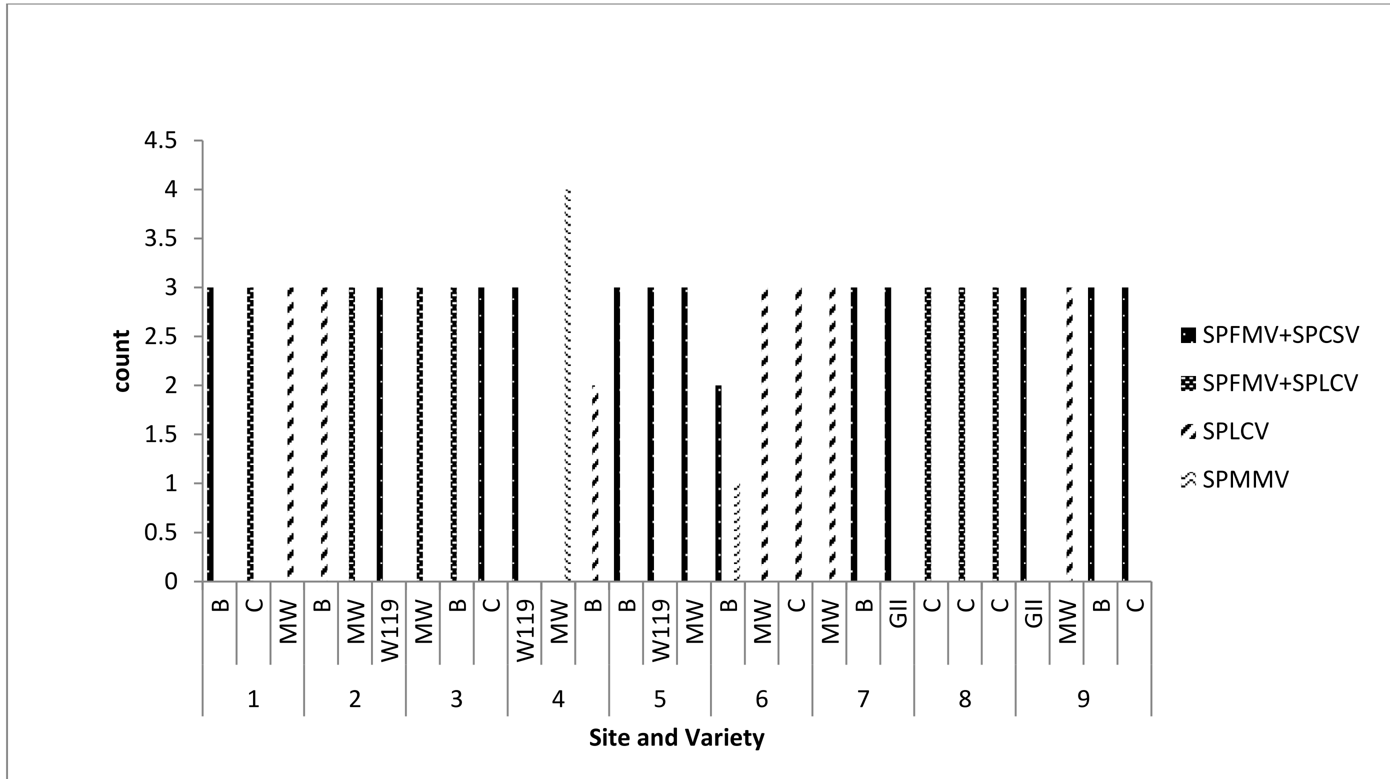


Figure 4.10: Incidence of viruses in different varieties of sweet potato and their distribution in different sites

4.1.3 Identification of viruses using the Polymerase chain reaction

RNA samples extracted from the 28 leaf samples from each site were separated into groups of three and run in separate trials, in the thermal cycler with 3 different primer sets for SPFMV, SPCSV and SPLCV. For all the three trials no bands were observed for all the samples as shown in Figures 4.11a-c.Only the DNA ladder bands were observed on the gel after viewing in the UV -trans illuminator indicating that PCR was unsuccessful. A positive control using maize ubiquitin gene primers was run in the same thermal cycler and no bands were observed on the gel after PCR (Fig 4.11(d)). Only the bands of the DNA ladder were observed.

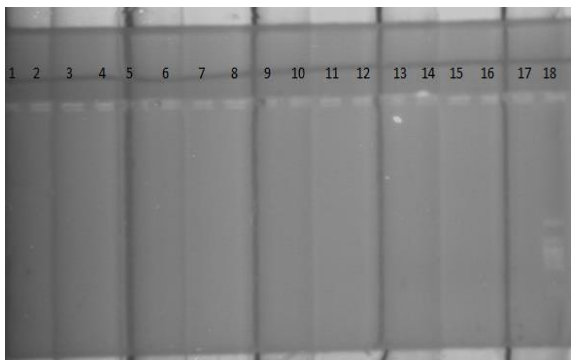


Fig4.11 (a):1st trial with SPFMV (F) (R)

Fig 4.11(b): Second trial with SPCSV(F)(R)

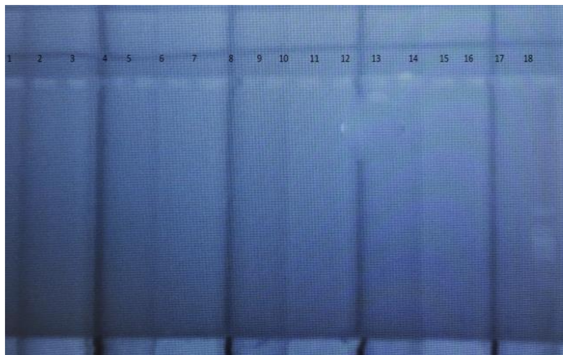


Fig 4.11(c): Third trial with SPCSV (F) (R)

Fig4.11(d): Positive control trial using Maize ubiquitin gene primers (F) (R)

4.1.4 Susceptibility of Chingovha, Mozambican White, German II, W119 and Brondal to SPFMV+SPCSV, SPFMV+SPLCV, SPLCV and SPMMV.

GII and W119 were the most susceptible varieties to SPFMV+SPCSV with a prevalence of 100% while MW was the least susceptible with a prevalence of 12.5%.Chingovha was the most susceptible to SPFMV+SPLCV and SPLCV with prevalence of infection ranging from 50%-57.1% while GII was the least susceptible to all the other viruses with a 0% prevalence. Highest prevalence of infection was recorded for MW at 12.5% making it the most susceptible to the virus.

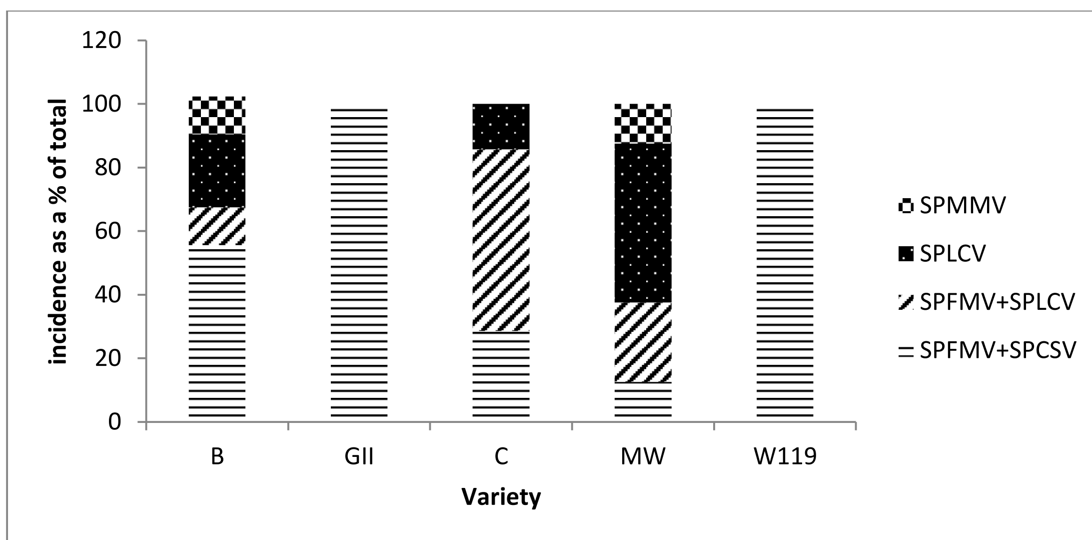


Fig 4.12 Susceptibility of varieties B, GII, C, MW and W119 to sweet potato viruses (SPFMV+SPCS, SPFMV+SPLCV, SPLCV and SPMMV).

4.2 Identification of fungi

Fungi were identified based on colony characteristics on PDA, structure of hyphae that are branched or septate and in some cases both, shape of conidia and spore arrangement. A total of four different taxa of fungi were observed in the 28 sweet potato samples collected from the nine sites. *Aspergillus* species and *Fusarium* species were identified up to the genus level while *Alternaria* species were differentiated into *Alternaria alternata*, *and A. bataticola* . Incidence of fungi ranged from 5-58 counts with *A.alternata* which was the most widely distributed having the highest incidence and being the most widely distributed while *Aspergillus* species had the least. Occurrence of the fungi was 5, 58, 9 and 20 counts for *Aspregillus, A. alternata*, *A. bataticola* and *Fusarium* respectively as shown in Fig 4.13(a).

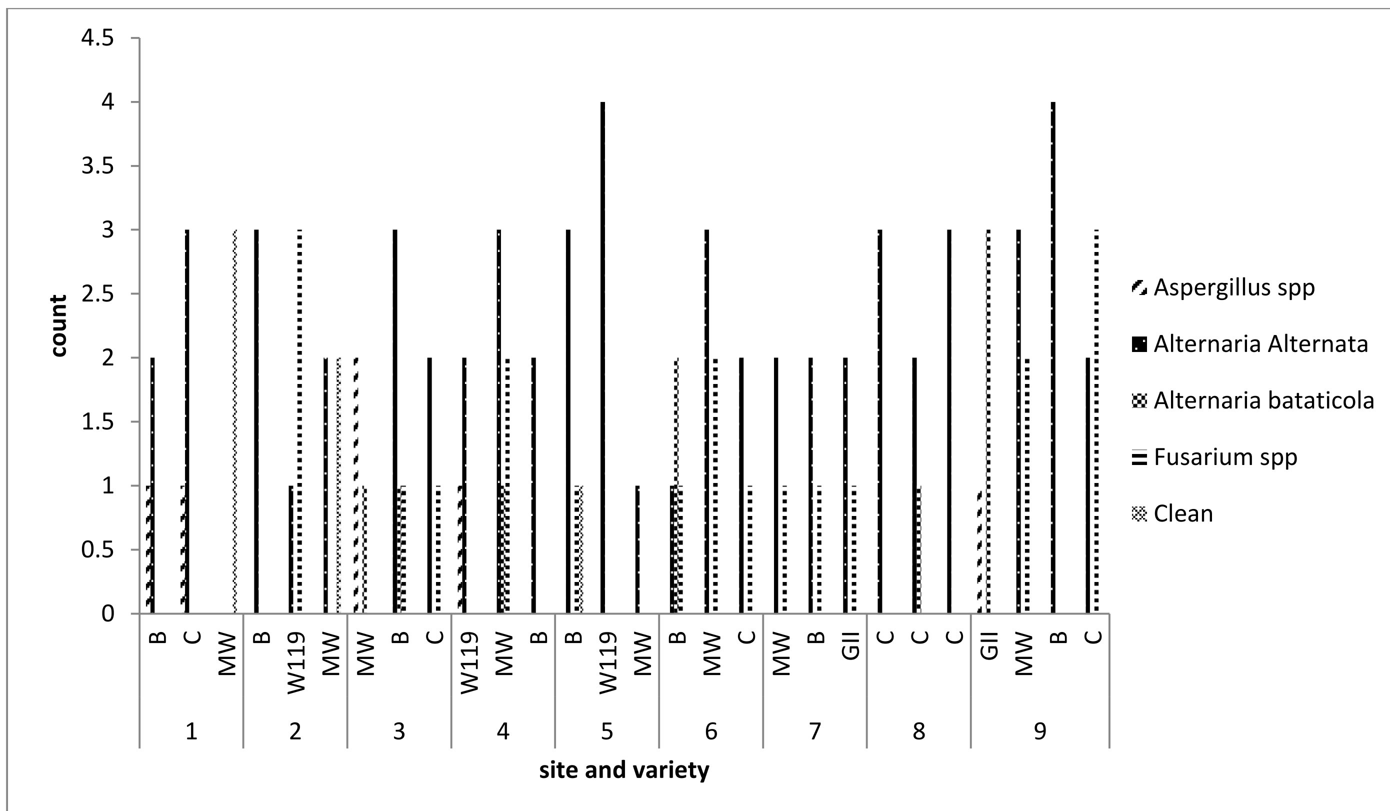
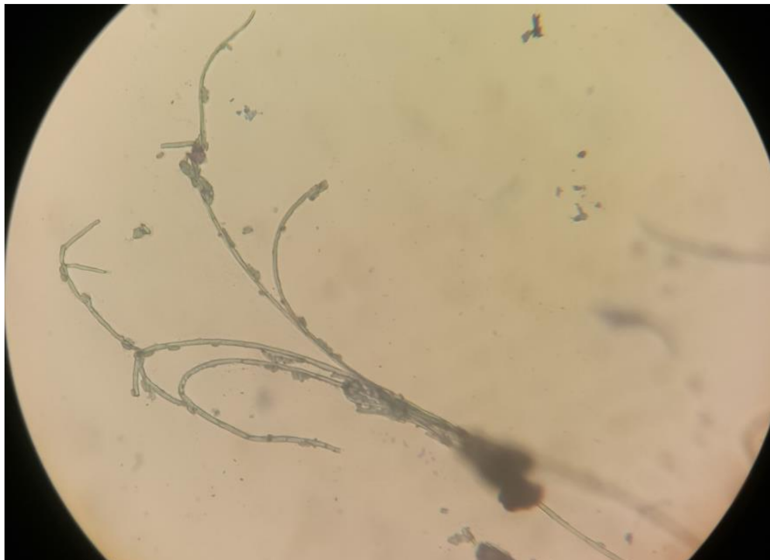


Figure 4.13 (a): Incidence of fungi in different varieties of sweet potato and their distribution in different sites

Aspergillus species observed produced greyish/green colonies on PDA plate's .Under the light microscope it exhibited branched septate hyphae with fruiting bodies that had a spherical form (Cleistothecia). *Alternaria* species typically produced fluffy pale brown to dark brown colonies on PDA. *Alternaria alternata* produced fluffy dark brown colonies with light brown edges. Under the light microscope conidia were ellipsoidal. *Alternaria bataticola* produced colonies with light brown centres and dark brown edges. Under the light microscope conidia of the fungus were solitary (isolated), obclavate and muriform. *Fusarium spp* isolates produced white aerial mycelia colonies on PDA .Under the light microscope conidia was oval to ellipsoidal shaped and not septate. Six plates did not exhibit any fungal growth. The control plate remained clean with no fungal growth or bacterial growth .**Fig 4.14(a)** shows a representative of how fungal characteristics were observed under the light microscope.



4.14(a): *Aspergillus spp* under the light microscope at x100

4.2.2 Susceptibility of B, C, MW, W119 and GII to Aspergillus spp, Alternaria alternata, Alternaria bataticola, Fusarium spp.

W119 was the most susceptible variety to *Aspergillus spp* and *A.alternata* with prevalence 20%-60% but the least susceptible to *A.bataticola* with 0% prevalence. MW was the least susceptible to *Aspergillus spp* with a prevalence of 6.66 % but the most susceptible to *Fusarium spp* with prevalence at 26.66% .GII was the least susceptible to *A. alternata* with 25% prevalence but the most susceptible to *A. bataticola* with 25% prevalence. B was the least susceptible to *Fusarium spp* with prevalence of 18.75%.Prevalance of infection in the 5 varieties was distributed as shown in Fig 4.15

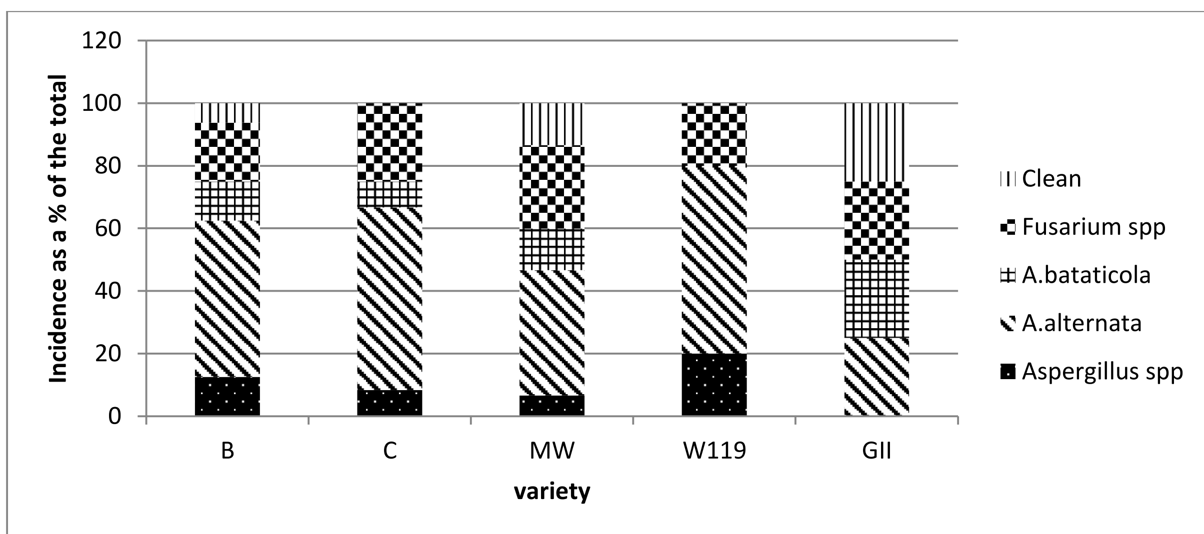


Fig4.15 Susceptibility of B, GII, C, MW and W119 to sweet potato fungi *(Aspergillus spp, Alternaria alternata, Alternaria bataticola, Fusarium spp).*

4.3 Association between viral species (SPFMV+SPCSV, SPFMV+SPLCV, SPLCV and SPFMMV) and fungal species *(Aspergillus spp, A. alternata, A. baticola, Fusarium spp*, Clean)

Associations between viruses and fungi were deduced from occurrence of viruses and fungi in different sweet potato varieties at the same time. In all sites, viruses identified were found occurring in synergy with fungi. Incidence of SPFMV+SPLCV occurring with *Alternaria alternata* and *Fusarium spp* was the highest (8 counts). There was spatial distribution of this combination of pathogens among six sites .SPFMV+SPLCV occurring with *Alternaria alternata* and *Aspergillus spp* had the second highest incidence (3 counts).This was the same incidence as in the case of SPFMV+SPLCV occurring with *Alternaria alternata* and SPLCV occurring with *Alternaria alternata* and *Fusarium spp*. Incidence of these combinations was distributed in three different sites. Other associations such as SPFMV+SPLCV occurring with *Alternaria alternata* and *Alternaria bataticola* had a lower incidence (2counts) in comparison to other associations. There was only one incidence of SPMMV was found occurring with *Alternaria alternata* and *Alternaria bataticola* and one incidence were there was no association at all between viruses and fungi.

CHAPTER 5: DISCUSSION

5.1 Occurrence of sweet potato viruses and susceptibility of varieties to infection

In Zimbabwe there is only one documented study by Chavi *et al.* (1997) on incidence and distribution of sweet potato viruses, which is why this study focuses on current incidence of viruses in Gweru, one of Zimbabwe's highly populated cities depending on sweet potato subsistence farming. Three viruses (SPFMV, SPCSV and SPMMV) out of the four viruses identified, were the same as those found infecting sweet potato varieties by Chavi *et al*. (1997) in 11 sites in Zimbabwe. The other virus (SPLCV) identified has never been documented before in Zimbabwe. SPFMV always occurred together with either SPCSV or SPLCV. It was never found infecting a sample on its own.

SPFMV+SPCSV complex had the highest incidence of infection (32 counts) while SPMMV had the least (5 counts). Prevalence of viruses tallies with prevalence of viruses identified by Chavi *et al*. (1997 who also documented high incidence of SPFMV+SPCSV and low incidence of SPMMV in the 11 sites he studied. The highest incidence of SPFMV+SPCSV in comparison to other viruses was also documented by Domola (2008) in the seven provinces he surveyed in South Africa. Sweet Potato Virus Disease leads to significant post-harvest tuber losses of up to 90% by inducing protein synthesis genes while suppressing photosynthesis and metabolism genes (Kokinos and Clark, 2006). Photosynthetic processes lead to tuber production so if photosynthesis is suppressed, tuber production and growth is marginal, if any at all even occurs (Kokinos and Clark, 2006). SPFMV +SPCSV complex was widely distributed occurring at eight sites. Extensive distribution of the viral complex might be a result of poor agronomy practices, where subsistence farmers in communities exchange infected planting material. Vegetative propagation of infected planting material perpetuates viral spread amongst varieties. Distribution of all virus infections among sweet potato varieties in different sites were a result of virus dispersal and morphology of the plant since all sites sampled have similar environmental condition. GII and W119 were the most susceptible to SPFMV+SPCSV while MW was the least susceptible. Leaf morphology determines susceptibility of varieties to infection (Kokinos and Clark, 2006). GII and W119 have shorter internodes and petioles in comparison to MW, which has longer internodes. The thicker leaf stem and stem cuticles found on MW samples make it difficult for whiteflies and aphid vectors to penetrate, making it less susceptible to infection by the viral complex (Kokinos and Clark, 2006).

SPFMV+SPLCV complex was not as common as SPFMV+SPCSV ranking second with 21counts. Chingovha was most susceptible to infection while GII and W119 were the least susceptible. Viral complex was not as common and was spatially distributed in four sites because SPLCV is not as common as the other viruses in Zimbabwe. Chavi et al. (1997) did not find SPLCV. Current incidence of this virus suggests that it might have been introduced into Zimbabwe possibly through sweet potato clones used in breeding programmes (Lotrakul and Valverde, 1999). Documented studies reveal that the viral complex leads to a yield reduction of up to 40 %, which is 50% less than that of SPFMV+SPCSV. These results are based mainly on the devastating effects that SPFMV has on yield since not enough data has been compiled on further characterisation of SPLCV and severity of its effects on yield. There was always high incidence of SPFMV in comparison with all other viruses in most documented studies of isolation of sweet potato viruses in Africa (Domola, 2008).

SPLCV occurred at six sites. Comparing distribution with that of SPFMV+SPLCV it seems SPFMV is spreading, infecting plants already infected by SPLCV. Data suggest that it is only a matter of time before samples with SPLCV also become infected with SPFMV. Increase in aphids in the different sites will accelerate spread of SPFMV to the plants already infected with SPLCV, leading to synergistic infections. On its own SPLCV leads to sweet potato leaf curl disease (SPLCD). Disease severity is still being studied but experiments carried out by Clark and Hoy (2006), show that variety infected by SPLCV yielded 24% less in comparison to other virus tested controls. MW was the most susceptible to SPLCV. Since leaf morphology of MW suggests that it is resistant to virus vectors, it is possible that during exchange of planting material, this variety was introduced into the environment already infected. GII and W119 were not susceptible to SPLCV at all probably because of their 100% susceptibility SPFMV+SPCSV leaving no room for other opportunistic infections.

SPMMV had the least prevalence and the most susceptible variety was MW.

5.1.2 Identification using PCR

There were no bands observed for the three RT-PCR trials using three primer sets for SPFMV, SPCSV and SPMMV detection. The only visible bands observed were bands of the DNA ladder Fig 4(a) (b) (c). Kwak et al. (2012) managed to identify SPFMV, SPCSV, and SPMMV using the same sets of primers as used in this experiment. Possible sources

of negative results were instrumental error, failure of primers to amplify the target sequences and lack of genetic material to be amplified in the first place. To investigate whether or not the thermal cycler was functioning properly a positive control using maize DNA and the maize ubiquitin gene F and R primers was run in the thermal cycler. Bands were only observed for the DNA ladder after running gel electrophoresis. Since there was no amplification of the maize ubiquitin gene using the F and R primers the thermal cycler was not working. Instrumental failure was the reason why there was no amplification of target sequences.

5.1.3 Comparison of reliability of methods used in identification of viruses

Observations in the field identified four viral diseases infecting sweet potato varieties in nine sites in Gweru. Results obtained from observations conformed to results obtained from the test plants. However SPMMV symptoms were observed, on a test plant inoculated with a B sample from site 6 which was only infected with SPFMV+SPCSV on observation. Inconsistency of results showed that there was possible cross contamination of test plants or a virus had been missed in previous observation. A control test plant showed SPMMV symptoms, three weeks after inoculation. The eyes missed symptoms that the sensitive indicator plant managed to identify. The SPMMV is sometimes symptomless and hard to identify by direct observation. *Ipomea indica* test plant proved to be more reliable in identification of viruses in comparison to direct observations. PCR was to be used as a standard to compare reliability of the two methods because of its specificity (Buckingham and Flaws, 2007) in identification of each of the viruses' genome but could not be done since PCR did not work.

5.2 Incidence of sweet potato fungi (*Aspergillus spp, A. alternata, A. bataticola, Fusarium spp*) and susceptibility of varieties to infection

Currently there is no documented study of fungi affecting sweet potato in Zimbabwe. The four types of fungi identified in this study were prevalent in all 28 samples. *A. alternata* had the highest prevalence with a widespread distribution in all nine sites. In healthy plants the fungi have minimal effects but in stressed plants the fungus leads to *Alternaria* leaf spot disease which reduces yield by up to 20%. Fungi are sometimes found occurring with *A. bataticola* as indicated by the results recorded in Appendix 1. The fungi lives saprophitically and dispersal of spores onto leaves of surrounding plants is dependent on the wind. W119 was most susceptible to *A. alternata* while GII was the least susceptible. Wind dispersal of spores as well as its ability to thrive on closely related

plants and weeds surrounding sweet potato encourages its wide distribution across sites. Dube, (2014) describes it as a relatively weak pathogen but it is also known as an opportunistic pathogen which reinforces why it is widely distributed and why most varieties are susceptible in all the nine sites investigated.

Second highest prevalence was recorded for *Fusarium spp* which had 20 counts. Fusarium species could not be identified to the species level in this study, but the most common species found in sweet potato is *fusarium oxysporum*. Agnes (2005) recorded high incidence of *Fusarium oxysporum* in sweet potato varieties in Kenya. *Fusarium* infections in sweet potato result in *Fusarium* wilt disease which results in yellowing of older leaves and transient wilting of the vines which eventually leads to vine death. Tubers infected if used as seed tubers infect fresh sprouts and lead to their wilting and death. Maximum damage occurs in sites were moisture content is low. This explains why there was widespread distribution of *Fusarium spp* in every site except for Site 1. Site 1 was located close to a water source so samples in this site were exposed to moisture. MW was the most susceptible variety with prevalence of about 27% and the least susceptible was B with a prevalence of 19%. There is no documented research as to what causes resistance of some varieties to *Fusarium spp* but it is possible that, high susceptibility of MW may be a result of it being in the field for a longer period. It would explain why over time it has been exposed to more fungi especially since fungi persist in the soil as resistant structures known as chlamydospores that can survive even after the crop has been wiped out in the field.

Incidence of A. *bataticola* was the third highest in comparison to the other four fungi species identified. *A. bataticola* was found occurring with *A. alternata* which is in harmony with observations reported by Anginyah (2000). *A. bataticola* leads to leaf petiole and stem blight diseases also known as Alternaria anthracnose when diseases are combined. Diseased plants face a yield reduction that ranges from 40%-90%. Double infection by leaf petiole and stem blight disease increases the devastating effects of the fungi. The fungi were spatially distributed in 5 sites with GII being the most susceptible while W119 was the least. Distribution of the fungus is encouraged by wind dispersal but sites in which the fungi are distributed are fewer than those of *A. alternata* because the host range for the fungi is only restricted to sweet potato. Some varieties such as W119 show resistance to infection by *A. bataticola* and resistance is a common phenomenon among certain varieties worldwide. Anginyah (2000) described resistance to *A. bataticola*

infection among East African sweet potato varieties. Further research still has to be done on the characteristics that all these varieties have in common that allow for this resistance. It could be that our own Zimbabwean W119 has resistant genes that allow for resistance or it could be that small and narrow leaf surfaces make it difficult for the spores to attach to the leaves.

Aspergillus spp which exhibit branched septate hyphae as one of their characteristics were similar to the *Aspergillus* identified by Oyewale, (2012). *Aspergillus spp* had the least prevalence amongst all the fungal species identified and there was sparse distribution of the fungi in four sites. *Aspergillus spp* owe their distribution to wind action which blows spores in dust particles which then comes into contact with the leaves (Oyewale, 2012). Some varieties are infected while others are not because even if the fungi is present in the environment, if variety is not susceptible the fungus is non-infectious and simply blown away to more susceptible varieties due to wind action. The most susceptible variety was W119 and the least susceptible was MW. Susceptibility of varieties to *Aspergillus spp* depends on their morphology and biochemical properties (Oyewale, 2012).MW is less susceptible because of its biochemical characteristics. Enzyme activity in this variety might be causes bio deterioration of the fungi. *Aspergillus spp* growth was high at 30°C in the incubator .The commonly identified *Aspergillus* species in sweet potato is *Aspergillus flavus*. As described by Oyewale (2012), in his investigation *A. flavus* growth and sporulation was optimal at 30°C which coincides with optimal conditions for *Aspergillus spp* growth in this investigation implying that there is a possibility that *A. flavus* was one of the *Aspergillus spp* identified. At 30°C optimal growth of fungi, aflatoxin production by the fungi was also high as described by Oyewale (2012). Aflatoxin is tolerable to the human being in small amounts but accumulation of aflatoxins from gradual consumption of infected sweet potato can present a health hazard. Oluwafemi (2012) detected different levels of aflatoxins in the breast milk of lactating mothers in western Nigeria communities were sweet potato was consumed as a staple food. Bio accumulation of the aflatoxins is a hazard to both the mother and the baby. Aflatoxins are carcinogenic and can eventually lead to development of cancers.

During inoculation perhaps there was uneven distribution of cell sap which resulted in fungal growth in some plates but no growth in replicates of those plates in which same media and same inoculum was used. It is also possible that absence of fungal growth in some plates could have been caused by their destruction during isolation process. There

was no fungal growth or bacterial growth in the control in which solvent used to solubilise cell sap was inoculated which shows that there was no contamination from unwanted microorganisms from the surrounding environment.

5.3 Association among viral species (SPFMV, SPCSV, SPLCV and SPMMV) and fungal species (*Aspergillus spp, A. alternata, A. bataticola and Fusarium spp*)

Results obtained show that there was an association in occurrence of fungi and viruses. Fungi –fungi interactions, virus-virus interactions and fungi –virus interactions have been reported before. Fitt et al. (2006) reported fungi-fungi interactions between *Fusarium* species and *Alternaria* species in sweet potato. Associations between sweet potato viruses are also very common. Cuellar *et al*. (2009) described the association between SPFMV+SPCSV as an RNase 3 protein mediated association. SPCSV RNase 3 protein targets specific host to synergize with by interfering with RNA biogenesis (Cuellar *et al*., 2009). Incidences of sweet potato viruses such as SPFMV occurring with Alternaria alternata have been reported by Clark *et al*., (2009). Incidence of SPFMV+SPLCV occurring with *Alternaria alternata* and *Fusarium spp* was the highest (8counts). There was spatial distribution of this combination of pathogens among six sites. Prevalence of fungal and virus complexes is different depending on geographical areas (Kuzdraliński et al.,2014) .In as much as incidence of complexes has been reported there is no conclusive research that explains sweet potato viruses and fungi relationship. High incidence of *A.alternata, Fusarium spp* and SPFMV+SPCSV complex shows that the pathogens can coexist within the host for a long time before one can lead to the extinction of the other. This relationship maybe explained by the synergistic antagonistic interactions concept (Andras *et al*., 2014). Sometimes when unrelated plant pathogens infect the same plant there is possible synergistic effects were incidence of a virus for example enhances the symptoms of fungus. This synergistic relationship can also explain the synergy observed between viruses and fungi in this research. Opportunistic fungi such as A.alternata can coexist with any pathogen as long as the host is vulnerable. *A.alternata* usually attacks sweet potato that is already deteriorating (Agnes, 2005). Where the virus does not occur with any fungi as reflected by the MW sample infected by SPLCV it is possible that the two pathogens compete for resources in the plant. The superior species wins the fight for resources so the other pathogen dies before it can spread within the plant.

5.4 Recommendations

Food insecurity, food insufficiency and poverty intertwined calls for implementation of effective strategies to tackle obstacles that stand in the way of production in Zimbabwe Viruses and fungi as identified in this study are the obstacles standing in the way of sweet potato productivity.

5.4.1 Recommendations to the farmer

Farmers are also encouraged to make use of virus free planting material for propagation. Virus free planting material is obtained from meristem shoot tip culture which can be used in combination with cryotherapy. Making use of virus free planting material can increase yields by up to 100% (Gao et al., 2000; Loebenstein et al., 2009). Farmers can employ biological control methods by use of natural enemies of virus vectors. Lady bird beetles are predators of aphids that spread SPFMV. Disinfecting soil can be difficult after removing infected plants from the site is because viruses and fungi can persist in the soil even after the crop has been destroyed. Integration of resistant cultivars into disease management practises can be very effective in curbing devastating effects of infection where soil disinfection cannot be done. Breeding programmes that result in resistant variety production can also be employed reduce susceptibility to infections.

Fungicides can also be used in the case of fungi, but they are a temporary measure because they are prone to weathering and breakdown over time. They only prove to be effective if used before infections become established.

5.5 Conclusion

All samples were infected with the viruses - Sweet Potato Feathery Mottle Virus (SPFMV), Sweet Potato Chlorotic Stunt Virus (SPCSV), Sweet Potato Leaf Curl Virus (SPLCV) and Sweet Potato Mild Mottle Virus (SPMMV). Samples were either infected by one of the viruses or a complex of two of the viruses. SPFMV always occurred in synergy with either SPCSV or SPLCV although SPLCV was sometimes found occurring on its own. SPMMV was always found in isolation. Occurrence of the viruses was in the ratio 38:21:20:5 for SPFMV+SPCSV, SPFMV+SPLCV, SPLCV and SPMMV respectively. SPFMV+SPCSV complex was the most widely distributed virus complex. MW variety was the least susceptible to devastating virus complexes such as SPFMV+SPCSV with an incidence of 12.5% but more susceptible to less harmful viruses such as SPLCV with an incidence of 50%. Widespread and spatial distribution patterns of viruses were possibly a result of

continuous propagation of infected planting material over time or presence or absence of viral vectors in the different sites. Different fungal colonies that indicated presence of *Aspergillus spp, Alternaria alternata, Alternaria bataticola* and *Fusarium spp* were observed on the PDA. Five MW plates and one B plate were free of fungal infection but exhibited bacterial growth. Prevalence of fungi was in the range of 5-58 counts for all varieties. Distribution of fungi in all nine sites sampled relies on dispersal mechanisms and moisture availability in the environment as in the case of *Fusarium spp*. Fungi identity was confirmed by viewing asexual reproductive structures of the fungi under the light microscope. GII and MW varieties were the least susceptible to fungi infections with prevalence of 25% and 13.33% respectively. Results obtained showed association in occurrence of viruses and fungi in sweet potato. This study has revealed that sweet potatoes in Gweru urban are infected with viruses and fungi, which can potentially lower yield by up to 90%. It is therefore imperative for the farmers to adopt control measures such as the use of virus free planting material and use of phytosanitation techniques to minimise post-harvest losses and eventually prevent it completely.

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APPENDICES

Appendix 1 Table showing incidence of viruses in sweet potato and their distribution in different sites

SITE	VARIETY	VIRUS			
		SPFMV+SPCSV	SPFMV+SPLCV	SPLCV	SPMMV
1	B	3	-	-	-
	C	-	3	-	-
	MW	-	-	3	-
2	B	-	-	3	-
	MW	-	3	-	-
	W119	3	-	-	-
3	MW	-	3	-	-
	B	-	3	-	-
	C	3	-	-	-
4	W119	3	-	-	-
	MW	-	-	-	4
	B	-	-	2	-
5	B	3	-	-	-
	W119	3	-	-	-
	MW	3	-	-	-
6	B	2	-	-	1
	MW	-	-	3	-
	C	-	-	3	-
7	MW	-	-	3	-
	B	3	-	-	-
	GII	3	-	-	-
8	C	-	3	-	-
	C	-	3	-	-
	C	-	3	-	-
9	GII	3	-	-	-
	MW	-	-	3	-
	B	3	-	-	-
	C	3	-	-	-
TOTAL		38	21	20	5

Appendix 2 Table showing incidence of fungi in sweet potato varieties and their distribution in different sites

SITE	VARIETY	FUNGI				
		Aspergillus spp	***Alternaria Alternata***	***Alternaria bataticola***	***Fusarium spp***	**Clean**
1	B	1	2	-	-	-
	C	1	3	-	-	-
	MW	-	-	-	-	3
2	B	-	3	-	-	-
	W119	-	1	-	3	-
	MW	-	2	-	-	2
3	MW	2	-	1	-	-
	B	-	3	1	1	-
	C	-	2	-	1	-
4	W119	1	2	-	-	-
	MW	-	3	1	2	-
	B	-	2	-	-	-
5	B	-	3	-	1	1
	W119	-	4	-	-	-
	MW	-	1	-	-	-
6	B	-	1	2	1	-
	MW	-	3	-	2	-
	C	-	2	-	1	-
7	MW	-	2	-	1	-
	B	-	2	-	1	-
	GII	-	2	-	1	-
8	C	-	3	-	-	-
	C	-	2	1	-	-
	C	-	3	-	-	-
9	GII	1	-	3	-	-
	MW	-	3	-	2	-
	B	-	4	-	-	-
	C	-	2	-	3	-
TOTAL		5	58	9	20	6

Appendix 3 Table showing melting temperatures of primers

Formula for calculation of melting temperatures

Tm = 4 (G+C) +2 (A+T)

Virus	Primer	Melting temperature
Sweet Potato Feathery Mottle Virus(SPFMV)	SPFMV (F)	56°C
	SPFMV (R)	56°C
Sweet Potato Leaf Curl Virus (SPLCV)	SPLCV (F)	66°C
	SPLCV(R)	64°C
Sweet Potato Chlorotic Stunt Virus (SPCSV)	SPCSV (F)	60°C
	SPCSV (R)	60°C
Sweet Potato Symptomless Virus(SPSMV)	SPSMV(F)	68°C
	SPSMV(R)	68°C
Sweet Potato Virus (SPV2)	SPV2(F)	62°C
	SPV2(R)	62°C

Appendix 4 Table showing associations between sweet potato viruses and fungi

	variety	Virus+ Fungi (incidence)
Site1	B	SPFMV+SPCSV+ Aspergillus spp + A.a
	C	SPFMV+SPLCV+ Aspergillus spp + A.a
	MW	SPLCV
Site 2	B	SPLCV+A.a
	MW	SPFMV+SPCSV + A.a
	W119	SPFMV+SPCSV + A.a +Fusarium spp
Site 3	MW	SPFMV+SPLCV+ Aspergillus spp + A.a
	B	SPFMV+SPLCV + Aspergillus spp + A.b
	C	SPFMV+SPLCV + A.a + A.b
Site 4	W119	SPFMV+SPCSV +A.a+ Aspergillus spp
	MW	SPMMV+ A.a+ A.b
	B	SPLCV+ A.a
Site 5	B	SPFMV+SPCSV+A.a + Fusarium spp
	W119	SPFMV+SPCSV+A.a
	MW	SPFMV+SPCSV+A.a
Site 6	B	SPFMV+SPCSV+ A.a+ A.b+ Fusarium spp
	MW	SPLCV+ A.a+ Fusarium spp
	C	SPLCV+A.a+ Fusarium spp
Site 7	MW	SPLCV+A.a+ Fusarium spp
	B	SPFMV+SPCSV+A.a+ Fusarium spp
	GII	SPFMV+SPCSV+A.a+ Fusarium spp
Site 8	C	SPFMV+SPLCV+A.a
	C	SPFMV+SPLCV+A.a+A.b
	C	SPFMV+SPLCV+A.a
Site 9	GII	SPFMV+SPCSV+Aspergillus spp+ A.b
	MW	SPLCV+A.a + Fusarium spp
	B	SPFMV+SPCSV+A.a
	C	SPFMV+SPCSV+A.a+Fusariumspp

Appendix 5: Table showing samples collected for each site and their varieties.

Site	Number of samples	Varieties
1	3	B ,C ,MW
2	3	B ,MW ,W119
3	3	MW ,B ,C
4	3	W119 ,MW ,B
5	3	B ,W119 ,MW
6	3	B ,MW ,C
7	3	MW , B ,GII
8	3	C ,C ,C
9	3	GII ,MW ,B ,C