Detection of plant pathogens associated with African nightshade seed and methods of seed processing to reduce infection

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Declaration

This thesis is my original work and has not been presented for the award of a degree in any other University/Institution.

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Dedication

To my late parents Peter and Monica Linguya, my guardian and mentor the late Godfrey Mwereria for their devotion to my education and moral support; my beloved wife Elizabeth Kananu and our children Emma Kathure, Emmanuel Mutugi and Ethan Mwongela for their patience, prayers and moral support that has made me reach this far.

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LIST OF ABBREVIATIONS AND ACRONYMS

AEZ-Agro Ecological Zone

AICAD-African Institute for Capacity Development

AIV-African Indigenous Vegetables

ANS-African nightshade

AOSA-Association of Official Seed Analysts

AVRDC-Asian Vegetable Research and Development Center

BLAST-Basic Local Alignment Search Tool

CABI-Centre for Agriculture and Biosciences International

CTAB-Cetyl trimethylammonium bromide

CAVS-College of Agriculture and Veterinary Sciences

CMV-*Cucumber mosaic virus*

CV-Coefficient of variation

DAS-Double antibody sandwich

DNA-Deoxyribonucleic acid

EB-Extraction buffer

ELISA-Enzyme linked immunosobent assay

FAO-Food and Agriculture Organization

IFPRI-International Food Policy Research Institute

IITA-International Institute of Tropical Agriculture

IPGRI-International Plant Genetic Resources Institute

ISTA-International Seed Testing Association

JKUAT- Jomo Kenyatta University of Agriculture and Technology

KEPHIS-Kenya Plant Health Inspectorate Service

KOH-Potassium hydroxide

LH-Lower highlands

LM-Lower midlands

LSD-Least Significant Difference

NCBI-National Center for Biotechnology Information

NGS-Next generation sequencing

PBS-Phosphate buffered saline

PCA-Principal component analysis

PCR-Polymerase chain reaction

PDA-Potato dextrose agar

PVY-*Potato virus Y*

RCBD-Randomized complete block design

RNA-Ribonucleic acid

RT-PCR-Reverse transcriptase polymerase chain reaction

SPSS –Statistical package for social sciences

TAS-Triple antibody sandwich

TMV-*Tobacco mosaic virus*

TOMV-*Tomato mosaic virus*

TYLCV-*Tomato yellow leaf curl virus*

TZ-Tetrazolium

UM-Upper midlands

UON-University of Nairobi

GENERAL ABSTRACT

African nightshade (ANS) is known for high micronutrient content, medicinal properties and fast growth with low production costs. The challenges facing its production include low quality seed, low leaf/seed yields per hectare, pests and diseases, poor harvesting and processing methods. The objectives of this study were to evaluate the quality status of African nightshade seed, detect seed borne pathogens, identify the most prevalent viral pathogens and determine a suitable seed processing method for clean ANS seed production. A household survey was conducted in 240 farms in Nyanza and Western Kenya where ANS is grown in abundance and seed samples were collected from farms and local markets. Certified seed samples were purchased for comparison. Quality and purity tests were done following International Standard Testing Association rules (ISTA). Purity was determined by separating seed samples into pure, discoloured, weed, other crop seeds and other foreign matter. Germination percentage was obtained by planting lots of 100 seeds on paper towel where number of germinated seeds, normal seedlings and infected seedlings were counted. Seedling vigour index was assessed by measuring seedling length and seedling dry weight. Fungal and bacterial pathogens were isolated and identified using appropriate methods to species level. Serology and molecular techniques were used to identify viruses and the major viruses were sequenced using next generation sequencing. Certified seeds were used as a standard check. The seed processing methods used by farmers were evaluated. Data was analyzed and treatment means compared using the fisher's protected LSD test at 5% probability level.

Seed quality tests showed that farm saved seed was of poor quality compared to seed obtained from the local market and certified seed. Farm saved and local market seed had low seed purity of 68.6% and 74%, respectively compared to certified seed at 94.4%. In addition, only certified seeds met the recommended moisture and germination percentage as per ISTA rules. There

was a significant ($p \leq 0.05$) correlation between seed quality and germination parameters. For example seed purity had significant positive correlation (r=0.76**) with germination percentage. The following major pathogens were detected in African nightshade: *Aspergillus flavus* (42.4%), *Aspergillus niger* (32.5%), *Xanthomonas campestris pv. vesicatoria* (31%), *Potato virus Y* (26%), *Penicillium chrysogenum* (23.5%), *Cucumber mosaic virus* (21%) and *Pseudomonas syringae pv. tomato* (17%). Three major viruses namely *Potato virus Y* (PVY), *Cucumber mosaic virus* (CMV) and *Tobacco mosaic virus* (TMV) were confirmed by next generation sequencing revealing genomic homology of more than 90% with 22 isolates from all over the world. Three strains of *Potato virus Y* were identified as PVY^{NTN}, PVY^{O} and $PVY^{N:O}$. Evaluation of seed processing methods revealed that wet seed fermentation method, produced seeds with the highest purity of 96.3% and yielded more (913.8 kg/ha) compared to other processing methods. The high level of ANS seed infection, contributes to poor seed quality leading to poor plant growth and low yields. There is need to train farmers on appropriate processing methods for clean seed production to increase ANS productivity. The current study recommends routine inspection of seeds by seed producers to ensure that the seed is of acceptable quality and within the tolerable levels of infection.

CHAPTER ONE: INTRODUCTION

1.1 Background information

The African leafy vegetables (ALVs) have been part of the food systems in sub-Saharan Africa for generations (Abukutsa-Onyango, 2003). They are particularly attractive to small scale farmers because they require relatively little external inputs; the risks of crop losses are much lower compared to the exotic vegetables, which typically require between 50 and 60% of variables in total costs (Abukutsa-Onyango, 2003). The average gross margin for ¼ ha of African nightshade (ANS) is estimated to be US$ 3,033 compared to US$ 1,760 for kale (Mumbi *et al*., 2006). The ALVs have high micronutrient content, medicinal properties, several agronomic advantages and economic value (AICAD, 2003).

African nightshade has become one of the most common vegetables in major supermarkets and green grocery stores in most African countries (Abukutsa-Onyango, 2003). A growing number of smallholders in turn are striving to take advantage of this renewed demand in the ANS by growing and supplying them to markets (FAO, 2010). However, farmers' capacity to meet a growing demand for these vegetables has been limited by lack of good quality seed. In Africa, less than 10% of the seed planted is purchased from the formal market (Weinberger and Lumpkin, 2007).

Farmers should produce ANS seeds which meet standards on purity, moisture content and viability (Mumbi *et al*., 2006). However, most farmers produce seeds that are associated with high seedling mortality and reduced yields (FAO, 2013). The ANS informal seed production system provides over 80% of the seed used by farmers (Mumbi *et al*., 2006). Farmers select and store part of their harvest for future planting, exchange seeds with relatives and other farmers or sell in local markets (IFPRI, 2012).

The crop is susceptible to pathogens that have a considerable negative economic impact on its health, leading to poor crop yields or no yield at all (Juliane *et al.*, 2015). Detection of diseases in plant material especially seeds is essential to ensure safe and sustainable crop production. The techniques available for pathogen detection have evolved significantly in the last few years to achieve rapid and reliable results (Jones, 2000). For example, serological and molecular techniques are the most commonly used methods today for detection of most pathogens (Juliane *et al*., 2015). In addition, these methods can also detect non-culturable and non-viable pathogens in plant tissues even when a high number of other micro-organisms are present (Albrechtsen, 2006). Polymerase chain reaction (PCR), a molecular technique, is presently the most widely used molecular technique for detection of plant pathogens (Carusso *et al*., 2002). The advantage of this method is its specificity and rapidity. However, the results obtained depend on the design of the primers, amplification and hybridization protocols (Carusso *et al*., 2002). Production of ANS is also constrained by poor seed processing methods traditionally used by farmers (Ekhuya *et al*., 2018). There is need to use appropriate methods to ensure clean seed production for high productivity.

1.2 Statement of the problem

African nightshade faces major constraints during production which include poor seed quality, inadequate knowledge on seed processing, heterogeneity of seeds and diseases (IFPRI, 2012). The seed sector is mainly informal and most farmers produce their own seed which is usually of inferior quality with low germination rates (IFRI, 2012). In most instances, farmers are forced to use large quantities of seed to compensate for poor germination rates (Mumbi *et al*., 2006). Majority of farmers use seeds obtained either from farm saved seeds or from open air markets, which have problems of purity with low germination rates (FAO, 2010). Depending

on such inferior seeds means that there will be poor plant growth and hence low yields (Abukutsa-Onyango, 2003).

Farm saved seed used for planting is likely to have degenerated over time because of seed borne diseases which lowers the quantity and quality of the vegetables produced (Cervantes and Alvarez, 2008). Seed borne diseases cause severe yield reduction and lead to excessive use of chemical pesticides that are a threat to environmental health (Albrechtsen, 2006). In solanaceous crops, approximately 18 to 20% of plant pathogens are seed borne and have indirect effects, such as the cost of chemical applied to control their vectors and in production of pathogen free seed materials (Cetintas and Yarba, 2010). Seed pathogens are largely responsible for seed rot and seedling mortality (Albrechtsen, 2006). Infected seed may fail to germinate and the pathogen from the infected seeds maybe transmitted to seedlings and growing plants in the field causing disease and eventual death of upto 100% loss of take-off (Cervantes and Alvarez, 2008).

There is scanty information on the use of serology and molecular methods for detecting seed borne pathogens in ANS. However, in other solanaceous crops these methods have proved to be rapid and robust in the detection of seed borne pathogens in plants (Hull, 2009). Seed processing is key in production of quality seed and farmers use different methods to process seed (Colley *et al*., 2015). Poor seed processing methods in turn leads to low quality seeds with poor germination rates (Adam, 2005).

1.3 Justification

Consumption of African nightshade has increased tremendously over the last two decades in East Africa and in Kenya. For example, research shows that indigenous vegetables accounted for 30% of all vegetables sold (Abukutsa-Onyango, 2010). There is need to address problems of low production and improve the quality of farmer produced seed to increase productivity. The seeds should be of good quality and produced in adequate quantities to meet requirements of seed health and high germination percentage (over 85%). In addition, the seed should have good storage attributes and adaptable to the various agro ecological zones. Information on the status of farmer produced seed and seed borne pathogen constraints as well as the seed processing methods used are important in coming up with applicable standards for clean seed production (Barros, 2002). Quality clean seed of ANS will enhance productivity in the farmers fields.

Seed borne pathogens and the associated mycotoxins present a serious challenge to clean African nightshade seed production. These pathogens infect the plants beginning in the field and the incidence is even higher for stored seeds (Chowdhury *et al*., 2005). It is important to determine whether a seed lot is free from seed-borne disease or contains pathogens within the maximum acceptable limit. Seed borne diseases lead to crops losses and sometimes permanent contamination of soil. Detection of seed pathogens at initial stages of infection is important for purposes of management (Vaideni, 2002).

The enzyme linked immunosorbent assay (ELISA) is relatively easy to use, high in sensitivity and reliability (Albrechtsen, 2006). Molecular techniques based on amplification and especially PCR, have been used for isolation of the most important seed borne pathogens

(Gutierrez *et al*., 2013). These methods can be optimized for use in detection of seed borne diseases in African nightshade. Correct identification of seed borne pathogens is critical for purposes of management and production of clean seeds to increase yields. In addition, farmers use poor seed processing methods with majority fermenting and sun drying them for long periods. Exposure of seeds to the sun ultimately leads to high nutrient losses lowering their viability (Oiye *et al*., 2009). An effective and efficient method for seed processing will increase the quality and germination potential of seeds leading to increase in ANS yields and more profit to the farmer.

1.4 Objectives

1.4.1 General objective

The general objective is to reduce losses due to seed borne pathogen infection and poor quality seed and increase productivity of African nightshade through clean seed provision.

1.4.2 Specific objectives

i. To assess the quality of African nightshade (*Solanum spp.* Miller) seed produced by farmers in Western Kenya.
ii. To detect seed borne pathogens associated with African nightshade (*Solanum spp.* Miller) seed produced by farmers in Western Kenya.
iii. To identify the most prevalent virus infecting African nightshade (*Solanum spp.* Miller) seed produced by farmers in Western Kenya.
iv. To determine the effect of seed processing methods on quality of African nightshade (*Solanum spp.* Miller) seed produced by farmers in Western Kenya.

1.4.3 Hypothesis

i. African nightshade (*Solanum spp.* Miller) seed produced by farmers has low quality attributes.

ii. Seed borne pathogens are prevalent in the African nightshade (*Solanum spp.* Miller) seeds preserved by farmers in Western Kenya.

iii. There are no commonly distributed viral pathogens infecting African nightshade (*Solanum spp.* Miller)

iv. Seed processing methods have an effect on the quality of African nightshade (*Solanum spp.* Miller) seed produced by farmers.

CHAPTER TWO: LITERATURE REVIEW

2.1 Production of African Nightshade

2.1.1 Diversity of African nightshade vegetable

African nightshade belongs to the genus *Solanum* in the family Solaneceae. The family is made up of approximately 90 genera and between 2000 and 3000 species and is well distributed throughout the tropical and temperate regions of the world (Gaya *et al*., 2007). Most recent research reports indicate considerable diversity among the African nightshades due to variations in their growth patterns, flowering time, leaf sizes, shapes, colour, tastes (bitterness), and nutritional and nutraceutical value, as well as composition and quantities of anti-nutrient contents (Ojiewo *et al.*, 2013). The common species of African nightshade grown in Kenya are *Solanum scabrum* Miller (entire to sinuate leaf margins and mature berries which are dark purplish black in colour), *Solanum villosum* Miller susp. *miniatum* (entire, sinuate, sinuate-dented or dentate leaf margins and mature berries which are orange dull in colour), *Solanum villosum* subsp. *villosum* (finely lobed dentate leaf margins and mature berries which are orange dull in colour) and *Solanum sarrachoides* Sendtner (mature berries are light green in colour with clearly lobed dentate leaf margins which are densely pubescent) (Gaya *et al*., 2007).

African nightshade is grown in both high and lowland areas in most African countries (Musyimi and Muthoni, 2009). The consumption of African leafy vegetables (ALVs) in Eastern Africa has increased tremendously. In Kenya for example, research shows that ALVs now account for 30% of all vegetables sold and seed yields of the above species range from 1036-1320 kg/ha (Ojiewo *et al*., 2013).

2.1.2 Ecological requirements and botany of the African nightshade

African nightshade plant is an erect dicot with many branches, growing 0.5 to 1.0 m high and it is propagated through seeds. It requires annual rainfall of approximately 500-1200mm which should be adequate if no irrigation is used (Drescher *et al*., 2009). It requires optimum temperatures of 18 to 30^0C. The plants prefer full sunlight, but can grow in partially shaded areas. It grows in most soil types and prefers light, medium and heavy soils, rich in nitrogen, phosphorous and organic matter (Mwai *et al*., 2007).

African nightshade is a spreading and rounded annual herb that can grow up to 75cm in height. The stems are purplish green in colour, branching, round or angular, smooth or partially hairy and becoming woody with age. Leaves are greyish green in colour, simple, alternate, ovate, or ovate-lanceolate (Vanrensburg *et al*., 2012). Leaf hairiness is variable; however, the leaves are mostly hairy. The plant has slender taproot with a fibrous root system. The flowers are white with a yellow centre. Both male and female organs occur on the same plant. Seeds are 1.8 to 2mm long and are light brownish yellow or purple in colour. The seeds are borne in small berries, about 5 to 12mm in diameter, green when immature and turn purplish black at maturity. They are produced occasionally in small bunches (Plates 2.1-2.6) (Vanrensburg *et al*., 2012).

Plate 2.1 *Solanum villosum*

Plate 2.2 *Solanum scabrum*

Plate 2.3 *Solanum americanum*

Plate 2.4 *Solanum villosum* fruit

Plate 2.5 *Solanum scabrum* fruit

Plate 2.6 *Solanum americanum* fruit

Plate 2.1-2.6 African nightshade (*Solanum* species) (Ojiewo *et al*., 2013).

2.1.3 Production and utilization of African nightshade

African nightshade is propagated by seeds, which can be planted in a nursery or direct in the field. Organic manures are commonly used by the farmers although fertilizers can also be used especially those with high amount of nitrogen and phosphorous (Mwai *et al*., 2007). African nightshade is usually ready for harvest in four weeks after transplanting. The leaves are normally harvested using a knife or hand. Picking is done at weekly intervals and leaves can be sun-dried for preservation (Latif *et al*., 2009). The leaves of African night shade are eaten as a cooked vegetable, sometimes mixed with other vegetables (Kimiywe *et al*., 2006). Some varieties have a bitter taste and others have a "sweet" taste. Those with bitter taste are boiled

and the water is discarded while the fresh fruit is also eaten. African nightshade is widely used as a traditional medicine in Africa. The leaf extracts of *Solanum scabrum* are used for the treatment of diarrhoea, stomach ulcers, some eye infections and jaundice (Drescher *et al*., 2009). The leaves also contain high levels of vitamin A, B and C, phenolics and alkaloids (Gaya *et al*., 2007). A diet incorporating African nightshade is recommended for pregnant and nursing mothers as it is rich in iron (Ojiewo *et al*., 2013). The leaves contain 87.2g water, 1.0mg iron, 4.3g protein, 38 kcalories, 5.7g carbohydrates, 1.4g fibre, 20 mg ascorbic acid, 442 mg calcium, 75 mg phosphorous, 3660 µg ß-carotine, and 0.59 mg riboflavin per 100 g fresh weight (Abukutsa-Onyango, 2007).

2.2 African nightshade seed systems in Kenya

2.2.1 African nightshade seed production in Kenya

Farmers' capacity to meet the growing demand for indigenous vegetables is constrained by lack of good quality seed with less than 10% of the seeds obtained from formal seed sources (Ojiewo *et al*., 2013). There is need to produce seeds which meet standards on purity, moisture content and germination rate (FAO, 2010). A quality seed determines to a large extent the amount of harvest. The seed should be of the right quality and available at the right place for a farmer to use (ISTA, 2014). Seed production in ANS is affected by a number of factors which include physical injury of the crop during establishment, poor growing conditions during seed development and nutrition of the mother plant (Ojiewo *et al*., 2013). In addition, physical damage during production or storage, moisture and temperature during storage and maturity of the seed during processing leads to poor quality seeds with very low germination rates (FAO, 2013).

Quality seed should be produced under conditions where genetic purity is maintained, conditions of growth are optimal, with proper timing and methods of harvesting (Drescher *et al*., 2009). In addition, appropriate processing methods should be applied during threshing, cleaning and drying. It is also important to use appropriate seed storage and seed distribution systems to avoid seed contamination (Ojiewo *et al*., 2013).

2.2.2 Seed quality attributes and testing methods

Farmers use seeds either saved from a previous crop or purchased from local markets, which have problems of purity with mean germination rates rarely above 50% (Abukutsa-Onyango, 2003). International rules on seed testing (ISTA) recommends the methods to be used by all seed testing laboratories (including non-ISTA member laboratories) when testing seed for trade transactions and for the enforcement of national laws for the control of seed quality (ISTA, 2014).

Selection of a good seed should be based on the various quality attributes. These quality attributes include; genetic purity, physical purity, seed health, seed viability, seed vigour and moisture content (ISTA, 2014). Genetic purity refers to the trueness to type and is determined by the genetic make-up, seed size and bulk density of seeds (Milosevic, 2010). Moisture content test is used to determine whether the seed will retain its germination potential from harvest to sowing time. It is important to the farmer because any kind of damage reduces the possibility of good crop production. Digital grain moisture meter is used in determining the moisture content and the loss of weight of a sample dried under specific conditions is considered as the moisture content and is expressed as % of the initial weight. Moisture content is reported in % corrected to one decimal place (ISTA, 2014).

Purity analysis test assesses the cleanliness of the seedlot which must be without impurities such as broken seed, soils, chaff and weeds. Seed germination is the ability of the embryo to germinate and is affected by a number of different conditions (Milosevic, 2010; ISTA, 2014). Germination involves the reactivation of the metabolic pathways that lead to growth and the emergence of the radical/seed root and plumule/shoot. The fundamental conditions for germination to occur include the viable embryo, seed dormancy broken and presence of proper environmental conditions (ISTA, 2014). Germination test determines the emergence and development from the seed embryo of those essential structures which make up the seedlings and which indicate the ability to develop into normal plants (Milosevic, 2010; ISTA, 2014). The results of a germination test are given as percentage of number of normal seedlings, abnormal seedlings, hard seed, fresh ungerminated and dead seeds. The higher the percentage of the normal seedlings, the better is the field establishment (ISTA, 2014).

Seed viability is the ability of the embryo to germinate and is affected by a number of different conditions and it measures whether the seed is alive or dead. It is designed to estimate the percent viability of the seed sample and by inference the seedlot (Groot, 2004; ISTA, 2014). This rapid test was developed to assess the viability of seeds using Tetrazolium (TZ) salt. It is based on the principle that viable tissues actively respire with enzyme activity. The hydrogen released during respiration within the viable tissues of seed combine with the colourless Tetrazolium solution to form a red colour within the tissues (Groot, 2004).

Seed vigour according to ISTA rules is defined as the sum total of those properties of the seed which determine the activity and performance of the seedlot during germination and seedling emergence (ISTA, 2014). Seed health test is done to detect the absence or presence of micro-

organisms especially the pathogens (Osborn, 2010). Seeds are incubated under laboratory procedures and given the best possible conditions for the pathogens to manifest (Groot, 2004).

2.3 Seed health status of African nightshade seed

2.3.1 Pathogen infection in African nightshade seed

Seed provides optimal environment for pathogen establishment thereby determining the health status of seed (Kaur, 2010). Seed infestation/contamination involves the physical mixing of the seed with propagative organs of pathogens such as spores, sclerotium and nematode galls (Islam, 2006). The pathogen can infect the embryo; it can be found under the seed coat, in the endosperm or cotyledon or on the surface of the seed (Groot, 2004). The pathogens can infect the seed through the flowers, fruits, through the stigma, through the wall of the ovary and through wounds and natural openings (Kaur, 2010). There is also infection by physical contamination for example the pathogen can stick to the surface of the seed, and their structures may be found in seeds and in the soil (Groot, 2004). Seed borne pathogens result in low germination of the seeds, discolouration and shriveling, death of crops and distribution of pathogen to new areas (Karavina *et al*., 2008). In addition, it may lead to introduction of new strains of the pathogen along with new germplasm from other countries and toxin production from the infected plants (Karavina *et al.*2008; ISTA, 2014).

Seed-borne diseases of economic importance include fungal, bacterial and viral pathogens. Seeds are carriers of these important diseases which lead to considerable yield losses (Lee *et al*., 2011). Fungi and bacteria not only cause seed deterioration, but also serve as sources of primary inocula of many diseases like seedling blight, damping off and wilts in nursery and fields (Hamin *et al*., 2014). Some of them are highly destructive, decreasing seed germination, causing seed rot, pre and post germination death (Islam, 2006). A considerable number of seed

borne fungi belonging to the genera *Aspergillus, Fusarium, Curvularia, Penicillium, Rhizopus, Colletotrichum* and *Macrophomina* has been detected in vegetable crops (Chowdhury *et al*., 2005). Bacterial diseases such as bacterial canker, bacterial speck, bacterial spot and bacteria wilt mainly affect the stems, leaves, roots or may be carried internally by the seeds causing considerable yield losses (Latif *et al*., 2009).

Plant viruses are also economically important pathogens in African nightshade production. More than 200 plant viruses are reported to be seed transmitted in one or more host species of solanaceous family crops and the number of reports on the seed-borne viruses continues to increase (Lee *et al*., 2011). Some of the common viruses in solanaceous crops include *Potato virus Y* (PVY), *Cucumber mosaic virus* (CMV), *Tomato spotted wilt virus* (TSMV), *Tomato mosaic virus* (ToMV), *Tobacco mosaic virus* (TMV), *Tomato yellow leaf curl virus* (TYLCV) and *Tomato leaf curl virus* (TLCV) (Hull, 2009). The management of virus diseases depends on proper identification of the virus and an understanding of the ecology and epidemiology of the virus transmitting vectors (Hull, 2009). When faced with a virus problem in vegetables, scientists may positively detect and identify the causal virus based on the symptoms and the use of diagnostic kits from commercial companies (Owolabi and Taiwo, 2001).

2.3.2 Epidemiology and description of important viruses associated with solanaceous crop

The epidemiology of viruses encompasses those factors that influence the spread of a virus (i.e transmission, acquisition, reproduction) (Jacquemond, 2012). These factors affect the crop due to differences in varietal susceptibility, incidence of virus disease, temperature and transmissibility (Gray *et al*., 2013). When the virus is translocated to the progeny the next generations of plants are infected. *Potato virus Y* (PVY), for example is transmitted from one

generation to the next producing infected plants in the field with consequently much lower yield and this provides the main source of PVY inoculum (Handiseni *et al*., 2008). Removing symptomatic plants (roguing) is important in preventing the spread of the disease (Jacquemond, 2012). Restricting the number of field generations of crops will also prevent the bulking up of the virus inoculum. In addition, volunteer crops and weeds are reservoirs of virus inoculum to cultivated crops (Gray *et al*., 2013).

Potyviridae is the largest genera of viruses that attack plants. It contains six genera and about 200 virus species, most of which have a monopartite positive single strand (+) ssRNA genome. The largest genus is potyvirus which contains 128 approved species (Gray *et al*., 2013). All potyviruses are transmitted by aphids (Jacquemond, 2012). Traditionally, virus species and virus strains were defined on the basis of symptomatology, host range and serology. Sequencing has enabled molecular taxonomy in closely related species (Cuevas *et al.*, 2012). Using the amino acid sequences of the CP (coat protein) of the virus species, the genus potyvirus exhibit 38-71% similarity, while strains share 90-99% similarity (Adams *et al*., 2004). *Potato virus Y* have single flexuous rod shaped particle of a modal length of 740nm consisting of over 2000 copies of CP monomers with a genomic RNA polyadenylated tail at the 3'-end and a VPg at the 5'-end encoding ten functional proteins (Cuevas *et al*.,2012).

Potato virus Y exists as a complex of strains which can be distinguished on the basis of their biology (i.e symptoms they elicit on indicator plants), serology and genome sequence (Kerlan *et al*., 2011). *Potato virus Y* strains are generally divided into the following groups: PVY^{O} (ordinary), PVY^{NTN} (necrotic recombinant), PVY^{N} (veinal necrosis) and PVY^{C} (stipple streak strain) (Kerlan *et al.*, 2011). The general symptoms in indicator plants include mild mosaic, severe mosaic, mottle, chlorosis and necrosis (Cuevas *et al*., 2012).

Cucumber mosaic virus (CMV) belongs to the genus cucumovirus, family: Bromoviridae, is one of the most widespread plant viruses with extensive host range infecting about 1000 species including cereals, fruits, vegetables and ornamentals (Ali *et al*.,2012). The virus is readily transmitted in a non-persistent manner by more than 75 species of aphids (Ali *et al*., 2010). CMV is a multicomponent virus with a single stranded positive sense RNA. RNAs 1 and 2 are associated with viral genome replication while RNA 3 encodes for movement protein and coat protein. Numerous strains of CMV have been classified into two major subgroups (subgroup I and II) (Madhubala *et al*., 2005). On the basis of serological properties and nucleotide sequence homology, the subgroup I have further been divided into two groups (1A and 1B) by phylogenetic analysis (Roossinck *et al*., 2015).

Tobamoviruses have a very wide host range and can cause serious economic impact in many crops i.e. cucurbits, brassicas and solanaceous (Spence *et al*., 2001). The viruses in this genus can easily be transmitted mechanically, through seed and contact between plants, but is not transmitted by vectors. The debris can become the most important sources of inoculum in the fields (Kumar *et al*., 2011). Several tobamoviruses are seed-borne, which contributes to disease spread (Kumar *et al*., 2011). *Tobacco mosaic virus* (TMV) and *Tomato mosaic virus* (ToMV) infect several solanaceous species. Both viruses produce local lesions on *Nicotiana glutinosa* and *Nicotiana tabacum* and are differentiated based on the symptoms (Spence *et al*., 2001). Cross reaction between TMV and ToMV antisera limits serological differentiation of two viruses by enzyme-linked immunosorbent assay (ELISA) (Spence *et al*., 2001). RT-PCR multiplex is a simple and easy method to differentiate two closely related viruses like TMV and ToMV (Kumar *et al*., 2011). Availability of complete genome sequence of tobamovirus

helps in designing specific primers for different gene targets which also helps in multiplexing for differentiation of viruses (Kumar *et al*., 2011).

2.4 Diagnostic methods for detecting seed borne pathogens

2.4.1 Detection of plant fungi and bacteria

Early diagnosis of seed borne pathogens is important since infected seeds may appear symptomless. Traditional techniques for detection of seed borne pathogens are based on incubation and grow-out methods (Chen *et al.,* 2007). Some pathogens can cause symptoms on seeds that are visible to the naked eye though for proper examination, a stereoscopic microscope is used and has the ability to reveal the presence of spores on seed surfaces (Walcott, 2003). Microscopy also detects the morphological abnormalities in seeds and can show embryos infected or those pathogens adhering to the surface of the seeds (Lievens and Thomma, 2005). Inspection of dry seeds can reveal presence of fruiting structures of fungi and the effects of fungi on the physical appearance of seeds. Some of these fungal structures and discoloration or pigmentation is detected by naked eye or by use of optical lenses (Lievens and Thomma, 2005).

Grow-on test or seedling symptom test is another method used to detect seed pathogens. Pathogens are capable of attacking seeds, leading to the rotting of seeds, and produce symptoms or death of young seedlings (Lievens and Thomma, 2005). These effects can be seen if seeds are sown on suitable substrate or grown under environmental conditions which support expression of such effects (Mathur and Kongsdal, 2003). This test involves placing seeds between paper towels or on sand to determine the number of seeds showing infection. Colonization of seeds by heavy growth of fungi results to loss of germination, death of plants,

discoloration of the roots, cotyledons, coleoptiles, hypocotyls and leaves (Mathur and Kongsdal, 2003).

The most popular and frequently used method for detection of a great number of seed pathogens is incubation. Two methods blotter and agar plating are recommended by ISTA for routine examination of crop seeds infection (Mathur and Kongsdal, 2003). Blotter method is a simple and inexpensive way to detect seed borne pathogens that responds to sporulation while agar plate method detects and identifies seed borne pathogens through colony characteristics (Mathur and Kongsdal, 2003). Seeds can be incubated in Petri dishes that contain medium-impregnated filter paper (i.e. blotting) or different agar media, under conditions that promote pathogen growth. Selective or semi selective media are used to reduce contamination especially by saprophytic organisms (Walcott, 2003). Although these are frequently used because of their simplicity of application, they are time-consuming, require mycological skills, and may not be sensitive at low levels of pathogen load (Chen *et al.*, 2007).

2.4.2 Detection of plant viruses

Serology is a more effective and widely applied method for detection of plant viruses due to the availability of species-specific antibodies (Walcott, 2003). The immunological methods for the detection of seed transmitted pathogens are based on the use of monoclonal and polyclonal antibodies that specifically bind to a target antigen, allowing the pathogen to be detected by enzymatic conversion of substrates (Babu *et al.*, 2012). The most commonly used is the double antibody sandwich-enzyme linked immunosorbent assay (DAS-ELISA) (Ward *et al.*, 2004). Serological assays do not require pure isolations as they are applicable for both biotrophic and necrotophic seed borne pathogens (Ward *et al.*, 2004).

Tests for viral infection are crucial for providing adequate supplies of virus-free seeds or seeds with very low infection levels to avoid intra-national and international dispersal (Ahmed *et al*., 2013). The enzyme linked immunosorbent assay (ELISA) has become the principal method for virus detection because it is relatively simple to use, high in sensitivity, reliable and suited for large scale testing and amenable to partial automation (Albrechtsen, 2006). In addition, serology testing can be done on ungerminated seeds to determine the incidence of virus transmission through seed to seedlings (Ahmed *et al*., 2013). Individual seeds are assayed to determine if they are infected or healthy and the results can be quantified (Ahmed *et al*., 2013). In indirect tests, seed sample units (subsamples) of specific quantity are assayed to determine the presence or absence of the pathogen in the sample unit and therefore whether the lot is positive or negative (Ward *et al*., 2004).

2.4.3 Use of molecular techniques to detect seed borne pathogens

Today, new techniques based on DNA analysis have been used and proven to be efficient due to high specificity and sensitivity (Paylan and Gumus, 2013). Due to its great potential, over the past 20 years, many PCR-based assays have been reported for identification of seed borne pathogens (Ahmed *et al*., 2013). The molecular tools have promoted efforts to set up assays with specific technical aspects (e.g. specificity, sensitivity, robustness) and economical demands (e.g. short diagnosis time, high-throughput, minimum taxonomic expertise and minimum cost (Lievens and Thomma, 2005).

The most common technique is conventional polymerase chain reaction (PCR); others include nested PCR, multiplex PCR, real-time PCR and magnetic-capture hybridization PCR. PCR has the ability to detect low levels of target pathogens (Ward *et al*., 2004). It also enables easy quantification of pathogens on seeds and interpretation of results due to its ability to distinguish

between closely related organisms (Pryor and Gilbertson, 2001). Ultimately, PCR can be used for detection of all seed-borne pathogens and thus supersedes conventional detection methods (Paylan and Gumus, 2013). However, there exist compounds within the seeds that can inhibit DNA amplification, resulting in false negatives. The cetyl trimethylammonium bromide (CTAB) method can be applied with the addition of particular chemical and enzymatic treatments to overcome DNA amplification inhibition (Pryor and Gilbertson, 2001). Reagents can be added to PCR mixture buffers, to allow consistent amplification of the target DNA fragment from undiluted DNA extracts from seeds (Pryor and Gilbertson, 2001).

2.4.4 Metagenomics studies using next-generation sequencing (NGS)

In recent years, metagenomics studies using Next Generation Sequencing (NGS) methods have greatly increased available knowledge on viruses infecting a wide range of hosts including plants (Ho and Tzanetakis, 2014). With these methods, it is possible to identify and characterize pathogens at the molecular level without any previous sequence knowledge generating high volume data. NGS has a dynamic range that allows detection of sequences with very low abundance (Liu *et al*., 2014). NGS allows phylogenetic analyses of complete genome in order to obtain representative set of sequences reflecting the diversity of strains and geographical distribution (Liu *et al*., 2014). Sequence alignment are performed, gaps and missing data removed and rate variation among sites estimated using gamma distribution (Gutierrez *et al*., 2016).

Phylogenetic trees are constructed using the neighbor joining method and the likelihood improved by the nearest-Neighbor-Interchange heuristic. Phylogenetic relationships are presented as mid-point rooted trees with branch lengths proportional to the number of base substitutions per site (Coutts and Jones, 2015). Seed certifying entities use the NGS as a virus

diagnostic tool in the inspection of micro-plants *in vitro* due to its high sensitivity and usefulness in detecting novel virus species (Coutts and Jones, 2015). Recently, Ho and Tzanetakis (2014) showed that the NGS bioinformatics analysis can detect even a single read of viral origin in a database containing 25 to 30 million sequences. In addition, the RT-qPCR technique can be used as a diagnostic tool during field stage in certification programme since the costs are affordable for seed companies and government phytosanitary agencies; furthermore, this method only requires a basic nucleic acid extraction, is fast (2-3 hours) and easy to implement in any laboratory (Coutts and Jones, 2015).

2.4.5 Methods for managing virus diseases

The methods of controlling diseases include the use of resistant cultivars, practicing field hygiene, vector control, eliminating the alternative hosts and weeds that harbor the diseases (Kaur, 2010). Resistant plants reduce the multiplication of seed borne diseases and the yields are not affected as is the case with susceptible plants (Paylan, 2013). Disease exclusion by planting certified seed and avoidance of vectors are the best strategies for disease management (Latif *et al*., 2009). Farmers should be able to recognize symptoms of virus infection in crops and rogue the infected ones to reduce disease spread (Taiwo and Owolabi, 2004). These are susceptible varieties that allow disease to multiply with only mild symptoms. These "symptomless carriers" leads to build up of inoculum in the fields (Lee *et al*., 2011). In addition, practicing field hygiene helps to eradicate diseases in plants eliminating the inoculum in the field (Paylan, 2013).

Crop rotation should also be done to avoid the availability of the same host leading to increase in disease inocula (Pandey *et al*., 2008). Controlling vectors through chemical means or use of plant barriers is also an effective method of preventing disease transmission (Baldauf, 2006).

Plant barrier crops like rye, sorghum and wheat have been used effectively as barriers to aphids infecting potatoes that transmit *Potato virus Y* (Baldauf, 2006). Early detection of plant pathogens in seeds allows for timely development of control and management strategies that goes a long way in avoiding epidemics. It is also a means of checking the spread of many seed borne diseases and it ensures prevention of disease spread to new areas (Taiwo and Owolabi, 2004).

2.5 Harvesting and processing of African nightshade seeds

There are two main methods of processing seeds (McDonald and Kwon, 2004). A dry seeded crop has seeds enclosed in pods or husks that are usually dried on the plant. Processing of dry seeded crops involve harvesting, drying, threshing and repeated cycles of winnowing, screening and further drying (Ekhuya *et al*., 2018). There are only two vegetable plant families with wet seeded fruits: the solanaceae (include tomatoes, peppers and nightshades) and cucurbitaceae (include melons, squashes and cucumbers) (McDonald and Kwon, 2004). Wet seeded crop has seeds that are embedded in the damp flesh of fruits. Wet seeded seeds are processed by soaking in water, fermentation, rinsing, decanting and then drying. Wet processing is mainly used by farmers in processing African nightshade although the method needs to be improved for clean seed production (Ekhuya *et al*., 2018). The soft fruits are cut up, mashed and then fermented. After fermentation is complete, the seeds are washed to remove pulp, pieces of fruit, debris and low quality seed (McDonald and Kwon, 2004).

African nightshade seed has low germination due to inadequate removal of sugars and germination inhibitors present in the fruit caused by improper seed extraction (Abukutsa-Onyango, 2003). Proper processing of seed determines the quality of the seed, for example, sun drying improves germination rate, seedling vigour and overall germination percentage

compared to the shade dried seeds (Elizabeth and Adeniji, 2015). Farmers process their seeds using different methods for example some sun dry fruits, thresh/squeeze and winnow while others crush, soak and dry them in the shade (Ekhuya *et al*., 2018). Farmers select plants seed production and processed seeds are stored in plastic pots, tins, bottles or polythene bags (Mwai *et al*., 2007). Seeds can be stored for a period of 6-24 months depending on the species and prevailing environmental conditions. The main preservative used is wood ash and later the seeds are sold in small quantities in open air markets (Ekhuya *et al*., 2018).

Seeds should then be dried fairly quickly after washing since slow drying may result in molds growth or premature sprouting of the seed (Kiremire *et al*., 2010). Seeds should be dried in a climate-controlled environment and once they are dry, they should be cured for two weeks. Seeds are dried using silica gel which is an important desiccant (moisture absorbing material) (Oiye *et al*., 2009). Seeds loose viability and vigor during processing and storage mainly because of high moisture content (McDonald and Kwon, 2004). High moisture increases respiration which may raise temperatures killing the seed, molds develop at high moisture content and pests such as weevils can breed causing rapid destruction of the seeds. There is hence need to dry seeds at the correct moisture content (Kiremire *et al*., 2010).

CHAPTER THREE

QUALITY ATTRIBUTES OF AFRICAN NIGHTSHADE SEED PRODUCED BY FARMERS IN KENYA

3.1 Abstract

Seed is an important input in crop production that should be of high quality to benefit farmers. This study was undertaken to evaluate the quality of African nightshade (*Solanum scabrum* and *S. villosum* Miller) seed produced by farmers in Kenya. A household survey was conducted in 240 farms using a semi-structured questionnaire to capture seed production systems and post-harvest practices that influence seed production. A total of 164 samples of farm saved, market and certified seeds were obtained during survey. Seed samples were tested for quality attributes and the Pearson's correlation between seed quality and germination parameters determined. The analysis showed that 50% of farmers use farm saved seed while 28% purchase seed from the local markets. Seed samples from different sources differed ($p<0.05$) significantly in quality attributes which are seed purity, moisture content, seedling vigor index and germination percentage. Farm saved and seed obtained from the local market had low seed purity of 68.6 and 74%, respectively, compared to certified seed at 94.4%. Certified seed had significantly ($p<0.05$) higher germination percentage compared to farm saved and local market obtained seeds. Only certified seeds met the recommended moisture and germination percentage as per the International Seed Testing Association (ISTA) standards. There was significant ($p\leq 0.05$) positive correlation ($r=0.76^{**}$) between seed purity and germination parameters. This study confirms that farmers are using low quality seed which raises the need to identify alternative ways of producing high quality seeds.

3.2 Introduction

African nightshade (ANS) has the potential to address food and nutrition insecurity, particularly for poor urban and rural households (Sthapit *et al*., 2008). It has a history of cultivation and domestication under African conditions, and the leaves and fruits being used as vegetables (Ambrose-Oji, 2012). The crop is an important source of micronutrients, fibre, vitamins, minerals and proteins (Gosh-Jerath *et al*., 2016). Majority of farmers either use seed saved from their crops, from neighbors or from local markets often with problems of both purity and germination (Abang *et al*., 2014). The absence of good quality seed leads to significant production losses affecting household incomes and food security (Sthapit *et al*., 2008). The informal seed acquisition in Kenya accounts for 90% of the African nightshade seed used by farmers (Gosh-Jerath *et al*., 2016). Farmers do not use certified seed due to limited supply, high prices and lack of knowledge on its importance (Sthapit *et al*., 2008).

Planting of high quality seeds is the first step towards optimizing crop production. Selection of good seed should be based on various quality attributes including genetic and physical purity, seed health, viability, vigour and moisture content (ISTA, 2014). Seeds saved from a previous crop or from the local markets have problems of purity with mean germination rates rarely above 50% (Onim and Mwaniki, 2008). Continued cultivation of recycled farm saved seed leads to overall decline in seed quality due to poor handling and accumulation of seed borne diseases. There should be adequate supply of certified seed to prevent yield losses. The objective of this study was to assess the quality status of the African nightshade (*Solanum scabrum*; *S.villosum* miller) seed used by farmers in Kenya.

3.3 Materials and Methods

3.3.1 Description of sampling regions

Seed samples were collected from four sites where African nightshade is grown in large quantities as a food and cash crop. These sites are Suneka and Ogembo in Kisii, Lurambi and Amalemba in Kakamega, Kenya. Global positioning system (GPS) was used to locate the sites.

Table 3.1 Description of the sampling regions

	Suneka	Ogembo	Amalemba	Lurambi
Location	Latitude :0^0 40' 43.5" S Longitude: 34^0 42' 27.7"E	Latitude: 0^0 50' 18.8" S Longitude: $34^0$43'47.6"E	Latitude: 0^0 16' 14.4" N Longitude: 34^0 45' 14.6" E	Latitude: 0^0 17' 42.5" N Longitude: 34^0 4' 47.9" E
Altitude	1500-2000 a.s.l. Upper midland zones AEZ (UM 2)	2000-2500 m a.s.l. Lower highlands zones AEZ (LH 2)	1300-1500m a.s.l. Upper midlands zone AEZ (UM 4)	1500-1900 m a.s.l. Lower midlands AEZ LM 2
Soil type	Well drained, dark-reddish brown, friable clay Chromoluvic phaeozems, partly pisofernic phase and mollic nitisols.	Well drained, Chromic vertisols and eutric planosols and chromic-luvic phaeozems	Well drained, dark-reddish brown, friable clay, with humic topsoil (basalts and nepheline phonolites).	The soils are well drained, moderately deep, dark red, friable clay (rhodic ferralsols, petroferric phase).
Rainfall	800-1000mm	1300-1600 mm	1000-1600 mm	1300-1500m a.s.l
Temperature	18-21^{o}C	15-18^{0}C	18-21^{0}C.	20-22^{0}C

Information source: FAO/UNESCO, 2000; Jaetzold *et al*., 2006.

3.3.2 Farm household survey and seed collection

A farm household survey and seed collection was done in August, 2017 after March/April long rains. Farm saved seeds used for testing seed quality attributes were randomly sampled from households who had some seed to sell or spare to be used for testing seed quality attributes. The respondents were selected using purposive sampling targeting farmers who grow African

nightshades. The survey was carried out to obtain household demographic information, African nightshade seed sources and understand farmers' post-harvest seed handling practices.

3.3.3 Sampling method and sample size

Data collection methods for this research involved the use of both qualitative and quantitative methods. The qualitative methods involved the use of key informant interviews while quantitative method was through administration of structured questionnaire and collection of ANS seed samples for quality testing. The tools for data collection during the survey included a structured questionnaire (Appendix 1) for collecting primary data and another set was used for key informant interviews (Appendix 2). Interviews and seed collection was carried out by selected and trained enumerators recruited from the local community with the help of agriculture extension officers. The questionnaire was pretested using a sample of 20 farms and revised accordingly. The targeted respondent was the household head, but in their absence, the spouse of the household head or a close relative or next of kin was interviewed. The key informant interviews targeted representatives from seed companies, seed stockists and government agencies.

The number of households to be interviewed during the survey was calculated using the formula adopted from Fischer *et al. (*1998) that is $\eta = z^2pq\ d^2$**.**Where η equals sample size, z equals standard deviation at the required confidence level (1.96), p equals the proportion of population tested at 0.05, q equals the proportion of the population not tested at 0.05 and d equals statistical significance at 0.05. Using the formula, the required sample size was 384 but due to the limited number and distribution of household growing ANS, only 240 farmers were interviewed (Table 3.2).

The population was stratified according to the agro ecological zones found within the study counties of Kisii and Kakamega in Kenya. Stratification ensured homogeneity within and heterogeneity among different strata. Proportional sample sizes were obtained for each of the stratum based on the target population of farmers associated with growing of ANS. The formula below was used: $\eta i = n/N*120$ where ηi equals sample size per AEZ, n equals total number of farmers in the AEZ and N equals the total number of farmers in the AEZ (Table 3.2).

Table 3.2 Number of farmers sampled in each agro ecological zone (AEZ)

AEZ	No. of farmers growing ANS	Sample size	Sample %
Suneka (UM2)	1230	58	24.2
Ogembo (LH2)	1320	64	26.7
Amalemba (UM4)	1440	72	30.0
Lurambi (LM2)	986	46	19.1
Total	4976	240	100

UM2: Upper midland zone two, LH2: lower highland zone two, UM4: upper midland zone four and LM2: lower midland zone two.

Proportional allocation was considered most efficient to make the probability of selecting a farmer in any strata to be equal and minimize variations within strata hence increasing reliability (Fischer *et al*., 1998). However, this was limited by numbers and distribution of farmers growing ANS. In addition to the above sample size, ten key informants were interviewed from research/extension (4), seed companies (2) and seed stockists (4). In addition, 120 farm saved seed samples were collected during the survey, 40 samples from the local markets and 4 from agro dealers (certified), which were used as a standard check. A standard weight of 50g per sample was maintained and samples were put in brown paper (khaki) bags that were stored at 5^0C at the University of Nairobi Plant Science laboratory awaiting seed quality tests.

3.3.4 Determination of seed purity and seed weight

From each seed sample, 30g were drawn and divided into 10g portions that were used in determining ANS seed quality. Seed samples were cleaned to obtain pure seeds, other crop seeds, inert matter (dirt), discoloured, shriveled and insect damaged seeds. Each component was weighed separately and the percentage fraction calculated as:

$$\frac{\text{Weight of each component}}{\text{Total test sample weight (10g)}} \times 100$$

The percent seed purity was compared with that recommended by the International Seed Testing Association (ISTA, 2014) for pure African nightshade seed: varietal purity (min %) =98% while analytical purity (min %) is 95%.

3.3.5 Determination of germination potential, viability and seedling vigor

From each seed sample, 30g were drawn and divided into 10g portions. Seeds were placed into containers and crushed into small pieces. Determination of seed moisture content was done using digital seed moisture meter (GMK-310 RT, G-Won Hitech Co. Ltd). The percentage moisture content was calculated by subtracting the weight of seed materials before drying (initial weight) and weight of seed material after drying (final weight) divided by initial weight of seed material and multiplied by 100. Three replications of 50 seeds from each source were distributed over blotting paper sheets, moisturized with an amount of water equivalent to 2.5 times weight of paper, inside plastic boxes (11.0x11.0x3.5 cm) and exposed to 20-30^{0}C with 8 hours of light and 16 hours of darkness. The evaluations on first and final germination percentages, seedling emergence, and seedling vigor index and seedling length were performed at 7 and 14 days in germination chamber after sowing in compliance with the rules for seed testing (ISTA, 2014). Seedling vigor index was calculated as seedling length (cm) x germination percentage (Dezfuli, 2008). The percent moisture content and seed germination

was compared with that recommended by International Seed Testing Association (ISTA, 2014) for African nightshade seed: Moisture content: Local (maximum-10%) while International (maximum-8%). The germination percentage: Local (minimum-70%) while International (minimum-80%).

3.3.6 Data analysis

Information from the survey questionnaire was coded on a numerical scale and entered into a spread sheet. The responses were summarized and similar responses combined, coded and analyzed using descriptive statistics, correlation analysis and multiple linear regression analysis (IBM® SPSS version 21, 2012). Data was presented using summary tables, charts and graphs. Pearson's correlation coefficients were determined for seed quality tests using Statistical Analysis System (SAS) version 9.2 (SAS; 2002). Means were compared using least significant difference LSD at $p \leq 0.05$.

3.4 Results

3.4.1 Demographic characteristics of African nightshade seed growing households

The survey established that 73.3% of the households were male-headed but 71.6% of the respondents were female. Half (50%) of the households sampled had between five to ten members with 65% being married. The distribution of the household head's level of education showed that 92.9% were literate. Fifty eight percent had primary school education; 31.3% with secondary education and 2.1% having tertiary education. Seven percent had no formal education. From the survey it was established that those who do farming as an occupation were 77.5% while small business enterprises were 6.3%, formal employees 5.4%, casual workers 3.3% and others 2.1% (Table 3.3).

Gender of the household head, gender of the respondents, age of the respondents and size of the household head had no significant correlation (Table 3.3). Marital status and occupation of the household head had significant negative correlation with other demographic information. The education level of the household head correlated positively with other demographic information (Table 3.3).

Table 3.3: Characteristics of respondents producing African nightshade in various agro ecological zones.

Description of variables	Variable	Valid percentages				Regression results	
		UM2 (%)	LM2 (%)	UM4 (%)	LM2 (%)	Mean & SD	EC
Gender of the (HH)	Male	71.1	72.5	73.8	74.6	176.8 (0.32)	Dependent
	Female	27.9	27.5	26.3	25.4	64.2 (0.218)	variable
Gender of the	Male	28.3	29.2	27.1	28.8	68 (0.22)	0.876
respondents	Female	71.7	70.8	72.9	71.2	172 (0.018)	0.893
Age of the household	21-30	2.5	2.92	3.3	2.92	7 (0.82)	0.84
head (HH) yrs	31-40	15	14.9	15.8	15	36 (1.26)	0.212
	41-50	22.1	22.9	24.6	22.9	55.5 (2.22)	0.163
	51-60	29.6	29.2	28.3	29.2	69.8 (2.51)	0.802
	Above 61	30.8	30.4	27.9	30	71.5 (3.11)	0.668
Size of the	1-5	30	30.8	29.1	31.3	72.75 (2.12)	-0.463
household(HH)	6-10	50.4	50	51.25	50.8	121.5 (1.29)	-0511
	11-15	15.4	16.5	15.83	14.58	37.25 (1.71)	-0.266
	Above 15	4.16	2.19	3.75	3.33	8.5 (1.29)	-0.168
Marital status	Married	65.4	65.8	65	66.7	157.8 (1.71)	-0.172
	Single	9.58	10	7.91	8.33	21.50 (2.38)	-0.078*
	Separated	19.6	19.2	20	20.42	47.5 (1.29)	-0.089*
	Widowed	5.41	5	7.1	4.58	13.25 (1.71)	-0.129
Education level of (HH)	None (illiterate)	4.58	5.4	6.25	5.83	13.25 (2.63)	-0.073*
head	Primary school	57.5	56.6	58.8	60.4	140 (3.92)	2.02 *
	Secondary School	34.6	35	31.25	30.42	78.75 (5.56)	0.01*
	Tertiary education	3.3	2.19	3.75	3.33	8 (0.82)	0.021*
Occupation of (HH) head	Farmers	76.3	75.4	77.08	78.3	184.3 (2.99)	-0.037*
	Formal employees	5.4	5.83	6.25	5.83	14 (0.82)	-0.058*
	Business persons	2.9	3.3	3.75	3.33	8 (0.91)	-0.046*
	Business persons & farmers	3.3	2.5	2.92	2.08	6.5 (1.39)	-0.022*
	Informal workers	6.7	5.8	5.42	6.25	14.5 (1.29)	-0.244*
	Casual workers	5.4	7.08	4.58	10 (4.17)	12.75 (3.1)	-0.269*

Mean and Standard Deviation [(SD) in parentheses]. All regressions are conducted using ordinary least squares estimations and estimated coefficients (EC) are reported. Asterisks on the coefficients denote the level of significance: *p<0.05, F-stat=6.68, R-squared=0.067.

3.4.2 Ownership and size of family land

The respondents cultivating African nightshade on ancestral land were 63.8% followed by those who had bought land at 23.8% and finally those that rented 8.3% (Figure 3.1)

The distribution of the farm sizes was mainly small scale with 51% of farmers having less than 2 acres and few farmers (0.1%) owned large parcels of land. However, majority of African nightshade farmers did it on a small scale (Figure 3.2).

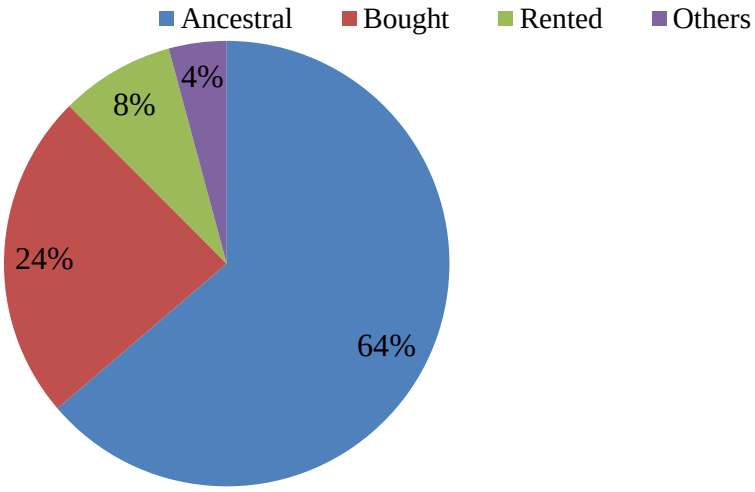


Figure 3.1: Type of land ownership

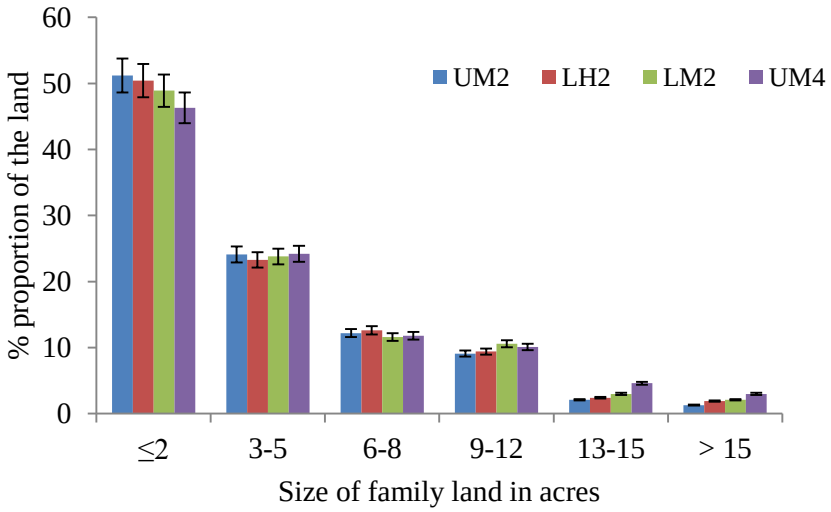


Figure 3.2 Size of family land in the major African nightshade production areas in Western Kenya.UM2: Upper midland zone two, LH2: lower highland zone two, UM4: upper midland zone four and LM2: lower midland zone two. Separation of means at $p \leq 0.05$.

3.4.3 Farmer preferences of Solanum species in different AEZs

Solanum villosum was the most preferred species of African nightshade in agroecological zones UM2 (83%) and LH2 (88%) while *Solanum scabrum* was the least prefered with UM2 18% and LH2 13% in Kisii. *Solanum scabrum* was the most prefered species in agroecological zones LM2 (78%) and UM4 (73%) while *Solanum villosum* was the least preferred in LM2 (20.8 %) and UM4 (22%) in Kakamega (Figure 3.3).

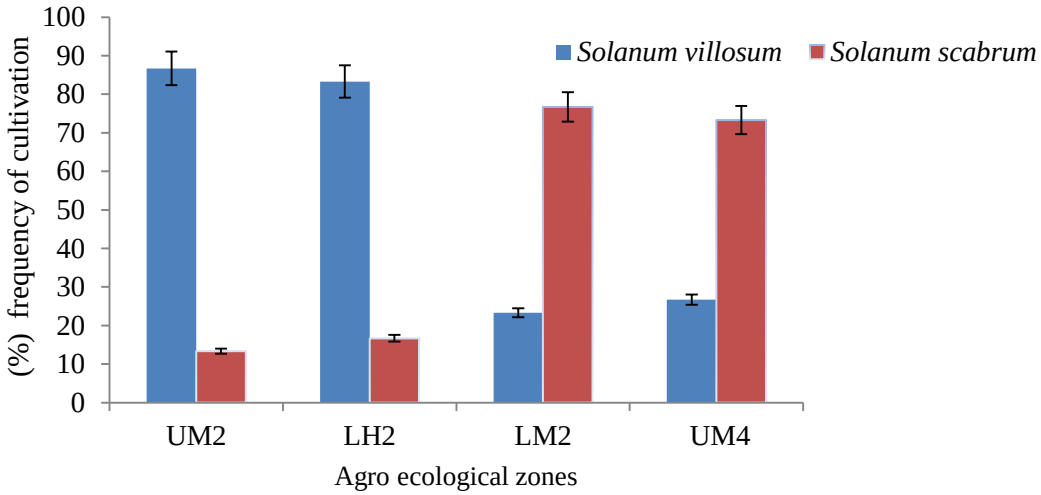
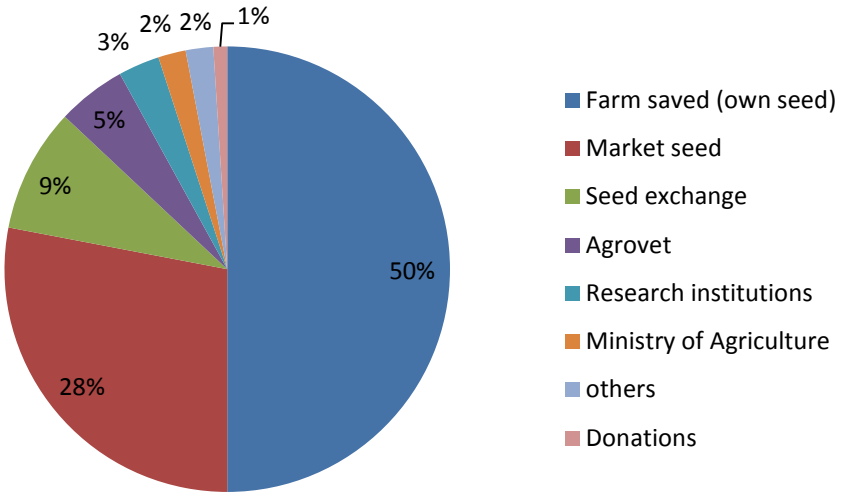


Figure 3.3: Farmer preferences of *Solanum* species in different agro-ecological zones
UM2: Upper midland zone two, LH2: lower highland zone two, UM4: upper midland zone four and LM2: lower midland zone two. Separation of means at $p \leq 0.05$

3.4.4 Seed sources of African nightshade

Fifty percent of farmers used farm saved seed followed by seed purchased from the market at 28%. Nine percent exchanged seeds compared to 5% who bought them from agro dealers. Three percent of African nightshade seed was obtained from research institutions and 2% from the Ministry of Agriculture (Figure 3.4).



Figures 3.4: Sources of African nightshade seeds in the Agro-ecological zones

3.4.5 Post-harvest seed handling by farmers

Sixty one percent of farmers reported to have observed pests and diseases on African nightshade seeds in storage. Slightly above a third (35.4%) used seed protection measures mainly dusting with ash for preservation. Farmers had varied responses on the seed harvesting state with 83.8% of them harvesting the seed when ripe followed by 14.2% who harvested when the seed was unripe, while 2% harvested when dry. After the seeds were harvested 53.3% farmers used wet processing followed by drying at 32.5%, while 14.2% did not extract seeds from plants and leave them in the field to grow. The seed was mostly stored in synthetic gunny bags (41.7%), with other storage materials being 21.3% gourds, 20.8% plastic cans, 10% paper bags and 6.3% earthen pots (Table 3.4).

The farmers knowledge on pests and diseases during storage, seed harvesting state and seed processing method were significant at $p<0.05$ in determining the seed quality (Table 3.4).

Table 3.4: Seed quality and post-harvest practices of African nightshade seed by the small scale farmers in different agro-ecological zones

Description of variables	Variable	Valid percentages				Regression values	
		UM2 (%)	LM2 (%)	UM4 (%)	LM2 (%)	Mean & SD	EC
Seed impurities (Seed quality)	Yes	15.4	15.8	18.3	18.8	41 (1.83)	Dependent
	No	84.6	84.2	81.7	81.25	199 (4.08)	variable
Pests & diseases in stored seeds	Yes	60.4	60.8	60	61.7	145.75 (8.71)	-0. 092*
	No	39.6	39.2	40	38.3	94.25 (6.023)	-0.082*
Use of seed protection measures to control pests and diseases	Yes	45.4	54.2	53.3	52.9	111 (9.83)	0.112
	No	54.6	54.2	53.3	52.9	129 (9.33)	0.724
Seed harvesting state	Ripe	83.75	83.3	82.5	82.9	199.5 (1.29)	0.045*
	Unripe	2.5	2.08	4.17	3.33	7.25 (2.22)	0.056*
	Dry	13.75	14.58	13.33	13.75	33.25 (1.26)	0.416*
Seed processing	Wet processing and drying	53.3	54.2	58.3	59.2	135 (7.02)	0.021*
	Dry processing	32.5	32.9	33.75	33.3	79.5 (1.29)	0.054*
	Unprocessed	14.17	12.9	7.92	7.5	25.5 (8.19)	0.058*
Drying method	Sun drying	98.3	98.75	98.3	97.9	236 (4.82)	0.866
	Shade drying	1.67	1.25	1.67	2.08	4 (0.82)	0.522
Seed storage	Gunny bags	42.08	41.7	42.5	42.92	101.5 (2.29)	0.99
	Gourds	20.8	20.42	21.25	21.7	50.5 (0.71)	0.21
	Plastic cans	20.8	20	19.6	19.2	47.75 (1.66)	0.28
	Khaki bags	2.92	2.5	3.33	2.92	7 (1.81)	0.36
	Earthen pots	6.7	5.8	7.08	7.5	16.25 (4.08)	0.33
	Polythene bags	6.7	9.6	6.25	5.83	17 (4.21)	0.190

Mean and Standard Deviation [(SD) in parentheses]. All regressions are conducted using ordinary least squares estimations and estimated coefficients (EC) are reported. Asterisks on the coefficients denote the level of significance: *p<0.05, F-stat=4.12, R-squared=0.056.

3.4.6 Correlation between level of education and African nightshade post-harvest practices

Farmers knowledge on the existence of pests and diseases in stored African nightshade seed, use of seed protection measures, existence of impurities in seeds, seed processing and storage methods were significant at $p<0.05$ with the level of education. In addition, farmers' level of education was not statistically significant with seed harvesting state and seed drying method (Table 3.5). The correlation coefficient for farmers' knowledge on the existence of pests and diseases (0.06), use of seed protection measures (0.08), existence of seed impurities on stored seeds (0.036), seed processing methods (0.024) positively correlated with respondents' level of education. However, the respondents level of education had a negative correlation with seed drying method (-0.08) and seed storage method (-0.172) (Table 3.5).

Table 3.5: Correlation analysis between education level of the respondents and post-harvest handling of African nightshade seed

Variables	Coefficient of variation	Standard error of means	*t*-Value	*p*-Value
Education level of the respondent	0.018*	0.003	1.22	0.048
Existence of pests and diseases	0.06*	0.092	-2.33	0.004
Use of seed protection measures	0.008*	0.001	2.13	0.046
Existence of seed impurities	0.036*	0.000	-2.03	0.006
Seed harvesting state	-0.878	0.375	2.81	0.667
Seed processing method	0.024*	0.003	0.060	0.032
Seed drying method	-0.08	0.005	1.070	0.448
Seed storage method	-0.172*	0.602	2.000	0.008

*Level of significance at p< 0.05

3.4.7 Seed Purity attributes

Certified seed was significantly ($p \leq 0.05$) higher in purity compared to seed from the local market and farm saved seed. Certified seed had more than 94% pure seed and least number of other crop seeds, inert matter, discolored seed and no shriveled seed nor insect damaged seed. It was close to the recommended 95% purity of the African nightshade seed (ISTA, 2014). Market and farm saved seed had higher percent of other crop seeds, inert matter, discolored seed, shriveled seed and insect damaged seed with low percent of pure seed and below the recommended seed purity by ISTA (Table 3.6).

Table 3.6: Mean (%) seed purity parameters of African nightshade seed from different sources

Seed sources	Pure seed	Other crop seeds	Inert matter	Discolored seeds	Shriveled seed	Insect damaged
[1]SS (market)	75.9[a]	5.2[b]	6.0[b]	5.6[b]	3.4[b]	4.0[b]
SS (farm saved)	69.6[a]	6.4[b]	5.9[b]	6[b]	5[b]	6.1[b]
SS (certified seed)	94.8[b]	2.0[a]	2.1[a]	1.0[a]	0.0[a]	0.0[a]
[2]SV (market)	74.0[a]	4.8[ab]	4.4[b]	5.8[b]	5.2[b]	5.8[b]
SV (farm saved)	68.6[a]	5.3[b]	5.6[b]	6.1[b]	7.3[c]	6.4[b]
SV(certified seed)	94.4[b]	2.1[a]	1.5[a]	2.0[a]	0.0[a]	0.0[a]
[3]Lsd ($p \leq 0.05$)	14.7	2.7	1.9	3.3	2.0	3.3
[4]CV%	29.2	27.3	34.2	32.2	31.8	32.2

[1]*Solanum scabrum*, [2]*Solanum villosum*, [3]least significant differences and [4]coefficient of variation. Values are the means, each having three replicates. Means followed by the same letter (s) within a column are not significantly different ($p \leq 0.05$). Means are separated by LSD at $p \leq 0.05$.

3.4.8 Seed germination parameters

Certified seed had a significant ($p \leq 0.05$) higher quality compared to market and farm saved seed. Farm saved and market seed had higher moisture content of above 10% while certified seed was less than 10%. Certified seed had the highest germination percentage of over 85% while farm saved and market purchased had low germination percentages of 72 and 68%, respectively. In addition, certified seeds had higher first count germination, seedling

emergence, seedling vigor index and seedling length, and significantly differed with farm saved and market seed which were lower (Table 3.7 and 3.8).

Table 3.7: Germination parameters of African nightshade seed from different sources in the first season

Sites	Seed source	MC (%)	FG (%)	FCG (%)	SE (%)	SVI	SL (cm)
UM2	Farm saved	12.4^{b}	66^{b}	44^{a}	74^{a}	211^{a}	3.2^{a}
	Market seed	12.1^{b}	68^{b}	53^{b}	75^{a}	252^{b}	3.7^{ab}
LH2	Farm saved	13.9^{bc}	69^{b}	51^{b}	76^{a}	242^{b}	3.5^{a}
	Market seed	10.0^{ab}	56^{a}	53^{b}	73^{a}	235^{a}	4.2^{b}
UM4	Farm saved	15.4^{c}	73^{bc}	59^{bc}	81^{b}	234^{a}	3.2^{a}
	Market seed	13.4^{b}	76^{c}	61^{c}	83^{b}	327^{d}	4.3^{b}
LM2	Farm saved	14.0^{c}	82^{c}	65^{c}	86^{b}	238^{ab}	2.9^{a}
	Market seed	11^{b}	75^{c}	62^{c}	84^{b}	285^{c}	3.8^{a}
	Certified seed	7.5^{a}	85^{d}	74^{d}	92^{c}	432^{c}	4.8^{b}
[1]Lsd p<0.05		2.6	8.6	6.1	5.4	24.3	1.2
[2]CV (%)		14.4	16.7	22.8	32.4	12.4	6.8

[1]Least significant difference. [2]Coefficient of variation. Means followed by the different letter (s) within columns are significantly different ($p \leq 0.05$); means are separated by LSD ($p \leq 0.05$).UM2-Upper midland zone two; LH2-lower highland zone two; UM4-upper midland zone four; LM2-lower midland zone two. Moisture content (MC), final germination (FG), first count germination (FCG), seedling emergence (SE), seedling vigor index (VI) and seedling length (SL).

Table 3.8: Germination parameters of African nightshade seed from different sources in four agro ecological zones in the second season

AEZ	Seed Source	MC (%)	FG (%)	FCG (%)	SE (%)	SVI	SL (cm)
UM2	Farm saved	12.3^{b}	69^{a}	54^{a}	68^{a}	193^{a}	2.8^{a}
	Market seed	11.1^{ab}	72^{ab}	48^{a}	72^{ab}	282^{c}	3.9^{ab}
LH2	Farm saved	12.3^{b}	71^{ab}	53^{a}	65^{a}	241^{b}	3.4^{ab}
	Market seed	11.2^{ab}	62^{a}	56^{a}	78^{b}	267^{bc}	4.3^{b}
UM4	Farm saved	15.6^{c}	76^{b}	55^{a}	74^{ab}	280^{c}	3.7^{ab}
	Market seed	14.4^{bc}	74^{b}	64^{b}	78^{b}	237^{b}	3.2^{ab}
LM2	Farm saved	13.0^{b}	79^{b}	68^{bc}	76^{ab}	260^{b}	3.3^{ab}
	Market seed	10^{a}	78^{b}	67^{bc}	83^{b}	297^{c}	3.8^{ab}
	Certified seed	8.3^{a}	88^{c}	75^{c}	95^{c}	422^{d}	5.7^{c}
[1]Lsd p < 0.05		3.1	10.4	9.3	8.2	29.3	1.3
[2]CV (%)		21.2	14.2	21.4	22.8	23.1	13.6

[1]Least significant difference. [2]Coefficient of variation. Means followed by the different letter (s) within columns are significantly different ($p \leq 0.05$); means are separated by LSD ($p \leq 0.05$). UM2-Upper midland zone two; LH2-lower highland zone two; UM4-upper midland zone four; LM2-lower midland zone two. Moisture content (MC), final germination (FG), first count germination (FCG), seedling emergence (SE), seedling vigor index (SVI) and seedling length (SL).

The proportion of germinated seedlings was higher in certified seed (96%) and differed (*P≤0.05)* significantly with 78% and 72% for market and farm saved, respectively. Certified seed had more than 95% non-infected seedlings compared to 76% and 72% for market and farm saved respectively (Figure 3.5). Farm saved seeds had the highest abnormal seed at 18%, 8% mouldy and 12% infected seedlings followed by market with 12% abnormal, 6% mouldy and 8% infected seedlings, while the lowest proportions were recorded in certified seed at 8% abnormal, 4% mouldy and 2% infected seedlings (Figure 3.5).

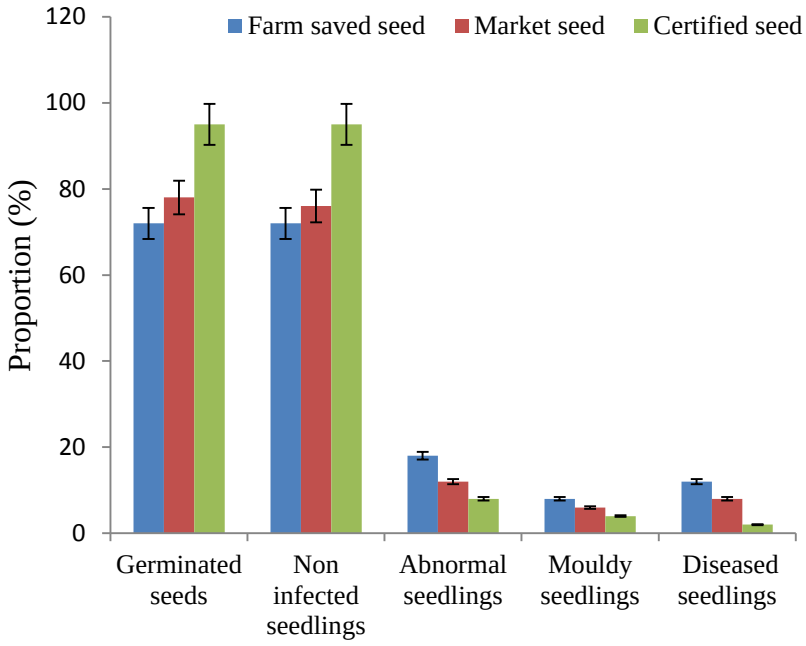


Figure 3.5: Mean proportions (%) of germinated, non-infected seedlings, abnormal, mouldy and diseased seedlings in seed samples. Separation of means at *p≤0.05*.

3.4.9 Correlation of seed purity and germination parameters

Seed purity showed a high significant positive correlation with germination percentage (r=0.76**), seed vigor index (r=0.76**) and seed weight (r=0.48*). There was non-significant correlation with seed moisture content and seedling length on farm saved seed.

Seed moisture content only correlated significantly with seed weight (r=0.56*) and had insignificant correlation with other parameters. Seed germination (%) had a high significant positive correlation with seedling emergence (r=0.63**), seedling length (r=0.68**), seedling vigor index (0.87**) and not significant with seed weight (r=0.32). Seedling emergence had a positive correlation with seedling length (r=0.54*) and seedling vigor index (0.64**) and non-significant correlation with seed weight (Table 3.9).

Table 3.9: Pearson's correlation (r) on seed quality parameters in African nightshade

Correlation Parameters	SP (%)	MC (%)	G (%)	SE (%)	SL (cm)	SVI	SW (g)
SP (%)							
MC (%)	-0.32^{ns}						
G (%)	0.76**	-0.04^{ns}					
SE (%)	0.28^{ns}	-0.24^{ns}	0.63**				
SL (cm)	0.22^{ns}	0.22^{ns}	0.68**	0.54*			
SVI	0.70**	0.27^{ns}	0.87**	0.64**	0.95**		
SDW (g)	0.48*	0.56*	0.32^{ns}	-0.02^{ns}	0.02^{ns}	0.36^{ns}	

SP: Seed purity, MC: Moisture content, G: Germination %, SE: Seedling emergence, SL: Seedling length, SVI: Seedling vigor index and SDW: 1000 Seed weight. *** Significant correlation at $p \leq 0.05$ and $p \leq 0.01$ respectively, ns: non-significant at $p \geq 0.05$.

3.5 Discussion

The study revealed that most of the respondents were women, which suggests that most men are engaged in other activities outside the farms. This implies that women are most likely the drivers of African nightshade crop production, recipients of agricultural extension messages and probably are decision makers. Similar findings were reported by Ogunlela and Mukhtar (2009) showing that women constitute over 60% of the agricultural work force and they play an important role in African leafy vegetables production. Most household heads were aged above 50 years hence the need for a strategy to engage youth in the production of African nightshade. Most farmers relied on agriculture as an occupation (77.5%) and the findings agree with the report by FAO (2013) that two thirds of farmers are dependent on farming for their

livelihoods. Majority of farmers cultivate African nightshade in small land holdings, which limits production due to competition for land.

Solanum scabrum and *Solanum villosum* are widely cultivated in Nyanza and Western Kenya because they are popular and preferred for it edible leaves and fruits. According to Manoko (2007), the two species are among the most intensively cultivated leafy vegetables. Farmers in these agro ecological zones prefer *Solanum scabrum* because of its ability to grow faster; it is well adapted to the climatic conditions in warmer humid agro ecologies and has large sized leaves that are good for consumption, lacking the bitter taste. Similar findings were reported by Matasyoh and Nyang'au (2016) in Kisii and Nyamira counties. Its seeds are also readily available in farms and market outlets. However, it was less cultivated in Kisii. Similar findings were reported by Olet *et al*. (2005) and Nyarango *et al*. (2008) that *Solanum scabrum* was absent in Kisii-Nyamira but was intensively and widely cultivated in Kakamega and Busia counties. *Solanum villosum* was the most preferred in Kisii UM2 and LH2 agro ecologies because it adapts well to the climatic conditions in the area, grows fast and the seed is easily availability (Olet *et al*., 2005).

Majority of the farmers reported that storage pests and diseases are observed in their seeds but only 35.4% of the respondents used crop protection measures. Farmers cultivate African nightshade for consumption and in the process collect seeds. In addition, more than 98% of farmers' sun dry their seeds leading to loss of quality as opposed to less than 2% who dry them under shade Babiker *et al.* (2010). The high temperature and U.V radiation accelerates respiration rate, causes seed breakage, bleaching, scorching and discoloration, damage to seed coat and loss of nutritional quality (FAO, 2013).

The age of the respondents and farmers level of education significantly correlated with postharvest practices in African nightshade seed production. Younger farmers were more aware of the existence of pest and diseases and seed impurities. However, older farmers processed and stored their seeds better. Farmers' knowledge of storage pests and diseases, use of crop protection measures, existence of seed impurities, seed processing and seed storage increased with the level of education. However, seed harvesting state, seed drying and storage methods had no significant correlation to the level of education. The result implies that relatively younger farmers are more dynamic in adopting measures to control post-harvest losses. In addition, an increase in the knowledge of farmers on post-harvest activities will bring a decrease in the post-harvest losses incurred. Similar findings were reported by Ali (2012) and Olayemi *et al.* (2012) while studying adoption of postharvest practices in vegetables.

This study has revealed that non-certified African nightshade seed saved by farmers and those sold in the market outlets are of low quality. The farm saved and market seeds failed to meet the recommended minimum standard for pure seed of 95% (ISTA, 2014). Market saved seed had less proportion of impurities compared to farm saved seeds; this could be attributed to the fact that farmers take more time to prepare seeds for market to attract customers. As expected certified seed had lower proportion of impurities compared to farm and market saved seeds. This could be attributed to good crop production and post-harvest seed processing as is done for other grains by the seed company that leads to quality seeds. The findings agree with those of FAO (2013) which reported low fraction of weed and foreign materials in formal seed compared to informal farm saved seed. In addition, Osborn (2010) reported that low purity levels in seed could be due to poor crop husbandry and post-harvest management practices (seed processing) by farmers such as stage of harvesting, threshing, drying and storage. The high proportion of discolored and shriveled seeds, abnormal, mouldy and infected seedlings in

the farm saved seeds compared to certified seeds could be attributed to high prevalence of seed borne diseases in recycled seeds leading to a build-up of inoculla. Similar findings by IFPRI (2012) reported that certified seeds are genetically pure and free from diseases, physical damage and immature seeds.

Certified seeds met the recommended minimum germination percentage of 85% and moisture content of less than 10% as per international rules of seed testing. Poor storage of farm saved seed increases seed moisture content (MC) which is known to reduce the longevity of the seed since any increase by 1% of moisture content can reduce seed storage life by approximately 50% (ISTA, 2014). The seeds should be adequately dried and stored in water-proof containers. Poorly stored farm saved seeds are exposed to storage pests and increases in moisture content, leading to reduction in seed longevity. Lower rates of germination, seedling vigor index and higher rates of moisture content in farm saved and market seeds could be attributed to poor pre and post-harvest handling and storage practices by farmers. Similar findings were obtained by Muthii (2014) and Stefano and Musya, (2010) who reported that threshing and other post-harvest processes by farmers lowers the germination capacity and seedling vigor. Low germination and seed vigor of farm saved seeds could also be due to long storage periods in poor conditions.

Warm and humid climate in Kisii and Kakamega counties and poor post-harvest handling practices by the respective farmers could have led to low seed quality due to high moisture content which predisposes the seed to infection. Farmers' harvest the crop under wet weather conditions and seeds are poorly dried leading to high moisture content (Gosh-Jerath *et al*., 2016). The end result is low seed vigor, poor germination and seed rotting. Seed quality had positive correlation with germination parameters showing that seed purity is a good predictor

of good crop performance. Similar results by Meseret *et al.* (2012) indicated that a pure seed has high germination percentage which results in a good crop stand.

CHAPTER FOUR

SEED-BORNE PATHOGENS ASSOCIATED WITH AFRICAN NIGHTSHADE (*Solanum scabrum* and *Solanum villosum* MILLER) SEED FROM DIFFERENT SOURCES

4.1 Abstract

Seed can be a carrier of important pathogens that lead to occurrence and spread of diseases with considerable yield losses. This study was carried out to determine the level of seed infection with plant pathogenic organisms in African nightshades. Seed samples from farm saved seed, seed purchased from the local market and certified seed were obtained from four agro-ecological zones in Kenya. Fungal pathogens were isolated using agar plating and blotter method, and identified using various methods. Bacterial pathogens were isolated by nutrient agar plating and identified through biochemical tests. The viral pathogens were detected using serology and molecular techniques. Laboratory experiments were carried out at the University of Nairobi and Kenya Plant Health Inspectorate Service (KEPHIS) from February 2017 to August, 2018. A total of seven fungal pathogens *Alternaria solani*, *Fusarium solani*, *Fusarium oxysporum, Aspergillus niger, Aspergillus flavus, Penicillium chrysogenum* and *Curvularia intermedia* were detected in the seeds. Two bacterial pathogens namely *Xanthomonas campestris* Pv. *vesicatoria* and *Pseudomonas syringae* Pv. *tomato* were detected. The viruses detected were *Potato virus Y, Cucumber mosaic virus, Tobacco mosaic virus* and *Tomato spotted wilt virus*. All the samples were negative for *Tomato yellow leaf curl virus* and *Pepper mild mottle virus*. The following pathogens had the highest frequency of detection: *Aspergillus flavus* (42.4%), *Aspergillus niger* (32.5%), *Xanthomonas campestris* pv. *vesicatoria* (31%), *Potato virus Y* (26%) *Penicillium chrysogenum* (23.5%), *Cucumber mosaic virus* (21%) and *Pseudomonas syringae pv. tomato* (17%). Farm saved seeds had the highest level of infection followed by market sourced seed, while infection was lowest in certified seed and differed

($p \leq 0.05$) significantly. Farmers should be sensitized on the need to plant clean seeds to improve crop yields.

4.2 Introduction

Farmers use African nightshade seeds saved from the previous crop or buy seed from the local markets which is of low quality (FAO, 2013). The seeds are infected or contaminated through physical mixing of the seed with pathogens propagation organs such as spores and sclerotia (Islam, 2006). The pathogen may infect the embryo, can be found under the seed coat, in the endosperm or cotyledon or on the surface of the seed. Some of the pathogens are known to infect the seed systemically through the flowers, fruits or funicules, the stigma, wall of the ovary or immature covers and through wounds and natural openings (Kaur, 2010). This leads to discoloration and shriveling, low germination and ultimately to development of plant diseases and spread of pathogens to new areas (ISTA, 2014).

Seed transmitted pathogens do not only cause seed deterioration, but also serve as sources of primary inoculum for many diseases like seedling blight, damping off and wilts in nurseries and fields (Chen *et al.,* 2007). Some of these diseases are highly destructive, decreasing seed germination and causing seed rot. In addition, plant viruses constrain the production of solanaceous crops especially tomato, pepper, potato and nightshade (Alvarez *et al*., 2005). Research on viruses in solanaceous crops has tended to focus on tomatoes and potatoes and little has been done on African nightshades. Viruses in the genera; potyviruses, tobamoviruses, begomovirus, cucumovirus and tospovirus have become a serious constraint in solanaceous crops production (Hull, 2009).

The most popular and frequently used method for detection of a great number of seed borne fungal and bacterial pathogens is incubation. Three methods namely standard blotter, potato dextrose agar and nutrient agar plating are recommended by ISTA for routine seed infection tests (Mathur and Kongsdal, 2003). The blotter method is simple and a cheaper way to detect seed borne pathogens that respond by sporulation while the agar plate method detects seed borne pathogens through colony characteristics (Mathur and Kongsdal, 2003). Serological and molecular techniques are the most accurate methods of detecting viruses in plant tissues and were employed in this study. Testing of seed for pathogen infection is critical in order to provide adequate supplies of quality pathogen free seeds to increase crop yields. The objective of this study was to determine the levels of seed infection of African nightshade seeds from different sources.

4.3 Materials and Methods

4.3.1 Description of sampling sites

Seed samples were obtained from four sites namely Suneka (AEZ UM2) and Ogembo (AEZ LH2) in Kisii, Lurambi (AEZ UM2) and Amalemba (AEZ UM4) in Kakamega, Kenya as described in section 3.3.1.

4.3.2 Seed collection and sampling procedure

Seed collection and sampling procedures were carried out as described in section 3.3.2.

4.3.3 Greenhouse experiment for seed-to-seedling transmission of pathogens

African nightshade seeds were planted in a greenhouse at the Department of Plant Science and Crop Protection, Field Station, University of Nairobi. African nightshade seeds were planted in 128-cell seedling trays containing commercial potting mix in an insect-proof greenhouse with temperatures ranging between 25 to 28^0C day/night and relative humidity of about 85%.

The experiment was laid out in a Complete Randomized Design (CRD) with seed from different sources replicated thrice. The seeds were sown on opposite sides of each cell 10cm apart to avoid disease transmission through leaf contact. In order to reduce the risk of cross-contamination among treatments from splash dispersal of diseases, careful manual watering was done. Seedlings were examined for symptoms of seed-transmitted infections on primary or first trifoliate leaves from 7 to 14 days post planting. In order to identify pathogens transmission through seeds, at least two seedlings from each pot were removed on the 14^{th} day and trifoliate leaves excised using sterile scissors and placed in sample bags which were stored at 4^0C awaiting pathological studies.

4.3.4 Isolation and detection of fungal pathogens from seeds

The agar plate and blotter methods were used to detect seed-borne fungi associated with African nightshade. Two hundred seeds in each seed lot were randomly selected, surface sterilized in 1% sodium hypochlorite solution for five minutes, rinsed in sterile distilled water and left to air dry. Seeds were plated in Petri dishes containing potato dextrose agar (PDA, Bioquest-Nairobi, Kenya) amended with 25 mgl^{-1} streptomycin sulphate. In addition, the standard blotter method was carried out for each seed sample using three layers of Whatman filter papers in a Petri dish and soaked in distilled water with 20 seeds of each sample being placed in rows. Petri dishes were incubated at 25^0C for 7 days under alternating cycles of 12 hours ultraviolet light (365 nm) and darkness. The experiment was set up in a completely randomized design with four replicates of five Petri dishes (10 seeds per Petri dish) and repeated twice.

Sections of the leaves excised from the seedlings were surface sterilized with 1% sodium hypochlorite solution for one minute. Leaves showing disease lesions were selected randomly

and a drop of distilled water was added over the lesion. Spores suspension were removed with a sterile pasteur pipette, deposited on the culture media, and spread over the surface of the medium with a sterile glass rod. The plates were stored in an incubator (Precision, Thermo Scientific, USA). The plates were incubated as described in section 4.3.4 following the two methods. After 7 days of incubation, fungal colonies grown on the nutrient agar were identified to species level using a stereomicroscope. Thereafter, selected fungal cultures were purified using the single spore technique.

Fungal isolates were sub cultured on PDA and incubated at 25^0C for 7 days under alternating cycles of near UV light and darkness. Morphological characteristics of each isolate were examined at 400x using a Zeiss (Munich, Germany) light microscope. The identity and incidence of fungal taxa isolated was determined and recorded. The reference manuals by Marthur and Kongsdal (2003) and Leslie and Summerall (2006) were used as a guide in identification of the fungi. Isolated fungi were stored on PDA agar slants at 4^0C. The percentage frequency of each fungal species was calculated by dividing the number of fungal isolates of individual species over the total number of all fungal species isolated from each sample and multiplied by one hundred (Leslie and Summerall (2006).

$$\text{Fungal frequency (\%)} = \frac{\text{Number of fungal isolates of individual species}}{\text{Total number of all fungal species isolated}} \times 100$$

4.3.5 Isolation and detection of bacterial pathogens

Bacterial isolates were obtained from African nightshade seeds and seedlings. The seeds were subjected to liquid assays as follows, one gram of seeds was surface sterilized with 3% (v/v) sodium hypochlorite solution for 4 minutes and thoroughly washed with sterile distilled water. Seeds were crushed using mortar and pestle in 5ml of phosphate buffered saline (pH 7.0) (PBS). Sections of the leaves excised from the seedlings were macerated with a disposable plastic

pestle. The macerates were suspended in 1 ml of PBS vortexed vigorously for 1 min followed by 10-fold serial dilution. The prepared suspensions separately for seeds and seedlings were left for 20 minutes and 10-fold serial dilutions were prepared and 0.05ml aliquots were spread onto triplicate plates of nutrient agar media with the help of spatula in a completely randomized design and repeated twice. Plates were incubated at 28 ± 2^0C for 24hr to 48hr. Single cell colonies were further isolated on the nutrient agar slants and stored at 5^0C for morphological studies. Colony size, shape, margin, elevation, texture, opacity, consistency, pigmentation and Gram staining were observed using slides at 600X magnification on a stereomicroscope. The morphological identification of bacteria was performed according to procedure by Marthur and Olga (2001) and Schaad *et al.* (2001). Bacterial isolates characterized using biochemical tests (Gram's staining, catalase test, levan formation, gelatin hydrolysis, starch hydrolysis, arginine dehydrolase, potassium hydroxide test, H_2S production and tobacco hypersensitivity reaction) according to Schaad *et al.* (2001) protocols. Single cell colonies were further isolated onto nutrient agar slants and stored at 5^oC for further studies.

Hypersensitivity reaction of bacterial isolates was tested on the foliage of tobacco plant (*Nicotiana tabacum* cv.Burley). Five isolates of each bacteria species obtained from seeds was used for testing. Test isolates were freshly grown on nutrient agar at 30 ± 2^0C for 24h and suspended in sterile water, maintaining an inoculum concentration of 10^8 cfu/ml (using Genie spectrophotometer at 550 nm). Tobacco seedlings were inoculated with bacterial suspensions of the selected isolates at 5 to 6 leaf stage. Bacterial suspension (1 ml) was injected into the intracellular space of the leaf on the lower surface with a hypodermal syringe and the plants sealed in plastic bags for 24hr to prevent desiccation. Plants were uncovered and kept in the laboratory at 28 ± 2^0C for 36hr. Control plants were injected with sterile distilled water. Hypersensitive response was observed daily and continued till the appearance of the symptoms.

4.3.6 Sampling for virus detection

A total of 120 samples were obtained from seeds, greenhouse and open field-raised seedlings. Forty samples from each category were used. One-hundred-milligram seeds and sections of the leaves excised from the seedlings of each seed source were crushed separately using sterile mortar and pestle in 100ml of PBS and preserved at -20^{0}C. To obtain leaf samples from the field, an area measuring 10m by 5m (quadrat) was selected to give a representative unit. In each zone ten farms growing African nightshade were selected. Top and middle leaves in ten symptomatic plants in each farm were taken at random and collected in small polythene bags (10x15 cm). Visual symptoms such as mosaic, leaf rolling, dwarfing, chlorosis, or a combination of these were assessed before sample collection and documented using a digital camera. Seeds and leaf extracts were stored at -20^{0}C in the molecular laboratory at Kenya Plant Health Inspectorate Service (KEPHIS) laboratory awaiting serology and molecular testing for viruses.

4.3.7 Serological detection of plant viruses

The following antibodies were purchased from the German collection of micro-organisms and cell cultures institute (DSMZ) in Germany and were used for DAS/TAS-ELISA (PVY: DAS:RT0343/PC0343,TMV:DAS:RT0041/PC0107,Potyvirus:DAS:RT0573/PC0573, CMV: DAS:RT0981/PC0981,TYLV:TAS:RT:0588-0546/2/PC-0588 and TSWV: TAS: RT-1154-1154/1/PC-0182). Buffers were prepared according to the manufacturer's instructions. The first antibody for detection of each of the six viruses in African nightshades was diluted in a coating buffer (coating buffer for 1 litre of distilled water; 1.59g Na_2CO_3; 2.93g of $NaHCO_3$; 0.20g of NaN_3; pH 9.6) and 100 µl were put in each well of ELISA plate. Samples were properly labeled and weighed. The plates were incubated for four hours at 37^{o}C after which the plates were

washed three times with the washing buffer (Appendix 2) and dried. An antigen extract 100 µl (0.6g plant material for 6ml of PBS+2% of polyvinyl pirroli) were added to the first antibody and the plate was incubated at 4^{o}C overnight. The second antibody was diluted (1µl in 1000 µl) to conjugate buffer (Appendix 2) and was added to the plate. After incubating for 4 hours at 37^{o}C, the plates were washed three times with washing buffer and dried before the substrate was added (Appendix 2) for luminofluorescence detection. The plates were incubated for 30 minutes at room temperature and absorbance determined using a microtiter plate reader (BioTeK El_x 800) at 405 nm. All samples were assayed in duplicates and results scored positive if the absorbance was greater than or equal to twice the average reading of the negative (healthy) controls.

Triple antibody sandwich (TAS- ELISA) was carried out according to manufacturer's instructions. The coating antibodies were diluted 100 times in coating buffer, while probe antibodies and the rabbit anti-mouse 1gG-AP were diluted 100 times in conjugate buffer. The steps including coating of the plates, loading of samples and overnight incubation in TAS ELISA were similar to DAS ELISA and were done as described in section 4.3.7. One hundred microliters of probe antibodies were added following washing step after overnight incubation. The plates were then incubated at 37^{0}C for 2 hours. The plate was washed again and 100µl rabbit anti-mouse conjugate 1Gg-AP (Alkaline phosphatase) was added before being incubated at 37^{0}C for 1 hour. A final wash was then performed and 100µl of substrate solution was added. This was followed with incubation at 37^{0}C for 30 min before observing and recording the results. All samples were assayed in duplicates and results scored positive if the absorbance was greater than or equal to twice the average reading of the negative (healthy) controls. The percentage frequency of each virus was calculated using the formula below: Percentage frequency = $\frac{\text{African nightshade samples confirmed +ve by ELISA}}{}$ X 100%

Total African nightshade samples tested

4.3.8 Molecular detection of viruses in African nightshade

Primer sequences were designed based on published sequence of genomes for each virus from the Genbank using Basic Local Alignment Search Tool (BLAST) provided online by the National Center for Biotechnology Information (NCBI, 2018). Primers were synthesized by Inqaba Biotechnology and Genomics Company following the sequences for each virus (Table 4.1).

Table 4.1: Nucleotide sequences of oligonucleotide primers used for molecular testing, genomic locations and target viruses in African nightshade.

	Primers	**Sequence (5'-3')**	**Product size**	**Target strain**
1	P12/3 P12/3A	ACAGCGTTTGGATCTTAGTAT GTGCGGTCTTAATAACCTCA	570 bp	Pepper mild mottle virus (PMMoV)
2	AV 494 AC 1048	GCCCTATAGTATCAGAAAGCCACAG GGGATTAGTGAGAGCATGTACGTACATG	550bp	Tomato yellow leaf curl virus (TYLCV)
3	CMV(F) (R)	GCCACCAAAAATAGACCG ATCTGCTGGCGTGGATTTCT	593bp	Cucumber Mosaic virus (CMV)
4	TMV(F) TMV(R)	CGATGATGATTCGGAGGC GAGGTCCARACCAAMCCAG	512bp	Tobacco mosaic virus (TMV)
5	L2-TSWV (F) L1-TSWV(R)	ATCAGTCGAAATGGTCGGCA AATTGCCTTGCAACCAATTC	364bp	Tomato spotted wilt virus (TSWV)
6	CP6P (F) CP6M (R)	CGTCCAAATGAGAATGCC TCTTGTGTACTGATGCCAC	577 bp	*Potato virus Y* (PVY)
7	S7P (F) S2M (R)	TTCCCAACAGGCGCAGTG CTAAACGGTCTGCCTTCAT	426 bp	*Potato virus S* (PVS)
8	X1P (F) X1M (R)	TCCTTATTCCAACGGCATC ATCTAGGCTGGCAAAGTCG	337 bp	*Potato virus X* (PVX)
9	n2258 (F) o2439c (R)	GTCGATCACGATGGATTTGGCGACC CCCAAGTTCAGGGCATGCAT	181 bp	$PVY^{N:O}$(necrotic recombinant-Normal & Ordinary strain)
10	o2172 (F) 02439c (R)	CAACTATGATGGATTTGGCGACC CCCAAGTTCAGGGCATGCAT	267 bp	PVY^{O}(ordinary strain)
11	s5585m (F) a6032m (R)	GGATCTCAAGTTGAAGGGGAC CTTGCGGACATCACTAAAGCG	452 bp	PVY^{NTN} (Necrotic strain)

Note: The full length primer sequences for specific virus were sourced from the NCBI nucleotide database and manually aligned in a text editor. The primer design software Prifi (Fredslund *et al*., 2005) was used to select primers.

4.3.8.1 Total RNA extraction

Total RNA was extracted from the leaves following the manufacturer's instructions (Qiagen): Liquid nitrogen was added to a sterile mortar containing frozen (200mg) leaf tissues which were ground thoroughly using a sterilized pestle. The ground tissue was transferred to an round-bottom microcentrifuge Rnase-free tube that had been cooled on ice. Lysis buffer of appropriate volume was prepared with 2-mercaptoethanol and added to each sample (1.5ml per 0.25g of grounded tissue). The lysate was homogenized by vortexing in order to disperse the sample, which was then incubated for 3 minutes at room temperature. A volume of 350µl lysate was transferred into a clean homogenization tube, and centrifuged at 13,000 rpm for 5 minutes. Ethanol (70%) was added to each volume of the homogenate. The sample was mixed thoroughly by vortexing in order to disperse any visible precipitate that formed after addition of ethanol. Up to 700 µl of the sample was transferred (including any remaining precipitate) to a spin cartridge (with a collection tube).

The sample was centrifuged at 13,000 rpm for 15 seconds at room temperature, the flow-through was discarded and the spin cartridge reinserted into the same collection tube. The 700 µl wash buffer was added to the spin cartridge. The sample was again centrifuged as in the previous step, but the spin cartridge inserted into a new collection tube 500 µl of washing buffer. Ethanol was added to the spin cartridge and centrifuged at 13,000 rpm for 15 seconds at room temperature, and then the flow-through was discarded. This last step was repeated. The spin cartridge centrifuged at 13,000 rpm for 2 minutes to dry the membrane with bound RNA. The collection tube was discarded and the spin cartridge inserted into a recovery tube. Fifty microlitres of Rnase-free water was added to the center of the spin cartridge and incubated at room temperature for 1 minute. The spin cartridge was then centrifuged for 2 minutes at 13,000

rpm at room temperature to elute the RNA from the membrane into the recovery tube. Finally the purified RNA was stored at -20^{0}C. This procedure was carried out for all the samples tested.

4.3.8.2 Extraction of viral DNA from plant tissues

Tomato yellow leaf curl virus (TYLCV) is a DNA virus from the genus Begomovirus. Genomic DNA was extracted from leaf/seed samples of plants using cetyl trimethylammonium bromide method (CTAB) as described by Gibbs and Mackenzie (1997). Samples were prepared by grinding 50mg fresh leaf/seed tissue homogenized in liquid nitrogen to a fine powder and 500 µl of CTAB buffer added to the powdered tissues. The mixture was centrifuged for 10min. The supernatant was removed and the mixture incubated at 60°C for 20 min. with gentle agitation. After the solution cooled down, an equal volume of chloroform: isoamylalcohol were added. The tubes were centrifuged at 3,000 rpm for 25 min at 10°C. The upper aqueous phase was transferred to a fresh tube and re-extracted with 2 ml of 10% CTAB and the mixture was incubated at 65°C. Chloroform: isoamylalcohol extraction was repeated and the mixture was centrifuged at 3,000 rpm at 10°C for 25 min. Two thirds volume of isopropanol was added to the upper supernatant phase in a fresh tube. The DNA was collected by centrifugation at 10,000 rpm for 20 min. The liquid was drained carefully and the DNA pellets were washed with 70% ethanol and the tubes were centrifuged at 5,000 rpm for 5 min.

DNA pellets were dried and re-suspended in 200 µl TE buffer. Four µl RNase A (10mg/ml) was added and incubated at 65°C for 1 hour. The DNA was precipitated again by adding 0.1 volume 3M sodium acetate and 0.7 volume isopropanol and left overnight at 4°C. The tubes were centrifuged at maximum speed for 15 min at 4°C and the DNA pellets were washed with 500µl 70% ethanol, centrifuged for 5 min. then air dried and re-suspended in 20µl of deionized and sterilized water. The nucleic acid was stored at -20°C.

4.3.8.3 Reverse transcription and polymerase chain reaction (PCR)

Total RNA for potyviruses, *Potato virus Y* (PVY), *Tobacco mosaic virus* (TMV) and *Cucumber mosaic virus* (CMV) was purified after extraction. Total DNA was used for complementary DNA (cDNA) synthesis using PCR kit (GoTaq, Promega, USA). cDNA was synthesized by mixing 5µl Dnase-treated total RNA, 2ml dNTP mix (10mM dATP, dCTP, dGTP and dTTP);1µl of 20 mM reverse primer and 2.25 ml RNase-free water in 0.5ml tube. The tube was then incubated at 65^0C for 5 min, chilled once, before adding 2ml of reverse transcriptase enzyme mix and 6ml RNase-free water. The tube was then incubated at 25^0C for 10 min, 40^0C for 30 min and 85^0C for 5 min. The synthesized cDNA was diluted 1:4 with ddH_2O and stored at -20^0C awaiting PCR. Polymerase chain reaction was used to amplify the 3' terminal genomic region of the virus using degenerate primers (Table 1). To detect potyviruses and PVY, the PCR master mix contained 2.5 µl cDNA template, 2 µl of each primer, 0.5 µl of 10mM dNTP, 10 µl of 10x Taq polymerase buffer, 0.2 µl Taq DNA polymerase (5 U/µl), 1.5µl of 25mM $MgCl_2$ and 31.5µl ddH_2O. The initial denaturation 2 min at 94^0C, 30 cycles of 94^0C for 30 seconds annealing at 57^0C for 1 minute. Initial extension was done at 72^0c for 1 min and the final extension was carried out at 72^0c for 5 min.

Cucumber mosaic virus PCR was performed in a 25-ml of reaction mixture consisting of 19.25 ml nuclear free water, 2.5 ml PCR buffer (100 mM Tris–HCl, pH 8.3, 500 mM KCl, 15 mM $MgCl_2$), 1 ml dNTPs (2.5 mM each), 0.5 ml forward and reverse primer (10 mM each), 0.25 ml Taq (5 U/ml), and 1 ml cDNA. The PCR reaction was carried out as follows: 94^0C for 2 min, followed by 35 cycles of 94^0C for 30 sec, 52^0C for 30 s, 72^0C for 45 sec, with an additional cycle of 72^0C for 10 min.

Tobacco mosaic virus PCR master mix contained 2.5 µl cDNA template, 2 µl of each primer (forward and reverse), 0.5 µl of 10mMdNTP, 10 µl of 10x Taq polymerase buffer, 0.2 µl Taq DNA polymerase (5 U/µl), 1.5µl of 25mM $MgCl_2$ and 32.5µl ddH_2O. The amplification conditions included an initial denaturation step at 94^0C for 2 minutes, followed by 30 cycles of 94^0C for 30 seconds (denaturation), 58^0C for 30 sec (annealing), 72^0C for 2 min (extension) and a final extension at 72^0C for 6 min.

Tomato spotted wilt virus (TSWV) PCR master mix contained 2.5 µl cDNA template, 2.5 µM of each primer, 0.5 µl of 10mMdNTP, 10 µl of 10x Taq polymerase buffer, 0.2 µl Taq DNA polymerase (5 U/µl) and 1.5µl of 25mM $MgCl_2$ .The amplification conditions included initial denaturation at 94^0C for 3 min, followed by 35 cycles of 94^0C for 60 seconds (denaturation), 52^0C for 30 sec (annealing), 72^0C for 1 min (extension) and a final extension at 72^0C for 6 min.

Tomato yellow leaf curl virus (TYLCV) each PCR reaction contained 2.5µl of extracted DNA, 0.25mM $Mgcl_2$, 1 ml dNTPs (2.5 mM each), 2.5µM of each primer, 1x enzyme buffer and 0.5µl of DNA TAG polymerase in 25µl final volume. The amplification reaction was carried out in a total volume of 25 µl using PCR thermal cycler, with denaturation at 94^oC for 30 sec, annealing at 50^oC for 45 sec, and extension at 72^oC for 1min. A single tailing cycle of long extension at 72^oC for 7 min was carried out in order to ensure flush ends on the DNA molecules.

4.3.8.4 Analysis of RT-PCR products by agarose gel electrophoresis

Amplified products of RT-PCR for each virus was examined on a 1% (w/v) agarose gel prepared in 1X-TAE buffer (Promega, USA) and stained with Ethidium bromide (1ug/ml). The 10µl for each product was added to 2µl DNA loading dye (Promega, USA). The GeneRuler^{Tm} 100 bp DNA ladder (Fermantas, UK) was used. Electrophoresis was done at 100V for 90min.

DNA bands were visualized under an ultraviolet (UV) transilluminator and photographed using a gel documentary system (Uvp's GDS 5000).

4.4 Results

4.4.1 Frequency of isolation of fungal pathogens from seed samples of *Solanum scabrum* and *S. villosum*

The fungal pathogens isolated from African nightshade seeds were *Alternaria solani, Fusarium solani, Fusarium oxysporum, Aspergillus niger, Aspergillus flavus, Penicillium chrysogenum* and *Curvularia intermedia* (Table 4.1). The frequency of isolation differed ($p \leq 0.05$) significantly among the fungal pathogens, plant species, AEZs and seed sources. *Aspergillus flavus* was isolated at the highest frequency of 26.5%, followed by *Fusarium solani* 26.2% while *Curvularia intermedia* had the lowest frequency at 3.1%. *Solanum scabrum* had a higher frequency of contamination with fungal pathogens compared to *S. villosum.* For example, the frequency of isolation of *Aspergillus flavus* from farm saved seed of *S. scabrum* was 40.6% compared to 26.6% in *S. villosum* with a similar trend being observed for all the fungal species (Table 4.2 and 4.3).

There were significant ($p \leq 0.05$) differences comparing fungal pathogens in the agro ecological zones, UM2 and LH2 zones in Kisii had slightly higher level of fungal pathogen infection compared to UM4 and LM2 zones in Kakamega. The three seed sources differed ($p \leq 0.05$) significantly with farm saved seed having a higher level of fungal pathogen infection followed by market seed and lowest in certified seed. For example the mean average of *Fusarium solani* in UM2 and LH2 was 37.5% in farm saved seed, 35.5% in market seed and 8.0% in certified seed (Table 4.2 and 4.3).Agar plating was more effective in detecting *Aspergillus niger, Aspergillus flavus* and *Penicillium chrysogenum* while blotter method was more effective in detecting *Alternaria solani, Fusarium solani, Fusarium oxysporium* and *Curvularia intermedia*

(Figure 4.1). There were significant ($p \leq 0.05$) differences in the level of pathogen infection relative to detection method, location (AEZ) and seed source (Table 4.4).

Table 4.2: Frequency (%) of occurrence of fungal pathogens in seed samples of *S. scabrum* and *S. villosum* from different agro ecological zones (UM2 and LH2) in Kisii county.

Seed sources	*A.solani*		*F.solani*		*F.oxysporum*		*A.flavus*		*A.niger*		*P.chrysogenum*		*C.intermedia*	
	UM2	LH2	UM2	LH2	UM2	LH2	UM2	LH2	UM2	LH2	UM2	LH2	UM2	LH2
S.s (Farm saved)	40.6^{a}	41.2^{a}	37.5^{a}	36.8^{a}	40.8^{a}	41.1^{a}	42.4^{a}	42.2^{a}	32.5^{a}	31.4^{a}	23.5^{a}	22.8^{a}	4.0^{a}	4.3^{a}
S.s (market)	41.5^{a}	40.6^{a}	35.5^{a}	34.2^{a}	41.6^{a}	40.2^{a}	43.5^{a}	44.1^{a}	31.5^{a}	30.4^{a}	22.6^{a}	21.6^{a}	4.1^{a}	4.1^{a}
S.s (certified seed)	10.0^{c}	11.0^{c}	8.0^{c}	7.2^{c}	7.2^{c}	6.8^{c}	6.8^{c}	6.4^{c}	11.4^{c}	10.6^{c}	3.8^{c}	3.7^{c}	1.0^{bc}	0.8^{bc}
S.v (farm saved)	26.6^{b}	24.2^{b}	22.4^{b}	23.2^{b}	21.0^{b}	29.2^{b}	28.5^{b}	27.8^{b}	23.8^{b}	24.2^{b}	14.6^{b}	14.4^{b}	1.5^{b}	1.8^{b}
S.v (market)	25.8^{b}	24.7^{b}	23.5^{b}	21.6^{b}	26.0^{b}	25.2^{b}	27.5^{b}	28.8^{b}	24.7^{b}	23.8^{b}	14.2^{b}	14.0^{b}	1.9^{b}	1.9^{b}
S.v (certified seed)	6.0^{c}	4.0^{c}	6.0^{c}	5.6^{c}	6.4^{c}	6.6^{c}	8.2^{c}	8.1^{c}	5.2^{c}	4.8^{c}	2.9^{c}	2.8^{c}	0.0^{c}	0.0^{c}
Lsd ($P \leq .05$)	12.0	11.8	11.2	9.8	11.2	10.9	13.3	12.2	6.5	5.8	7.2	6.9	0.8	1.2
CV (%)	33.1	29.2	31.7	28.6	26.7	27.5	32.6	28.8	19.1	20.1	33.3	32.1	24.3	23.2

Means followed by the same letter(s) within each column are not significantly different at $P \leq .05$, LSD - Least significant difference at $P \leq .05$, CV- coefficient of variation, Ss-*Solanum scabrum*, Sv-*Solanum villosum*, AEZ- UM2-Upper midland zone and LH2- lower highland zones.

Table 4.3: Frequency (%) of occurrence of fungal pathogens in seed samples of *S. scabrum* and *S. villosum* from different agro ecological zones (UM4 and LM2) in Kakamega county.

Seed sources	*A.solani*		*F.solani*		*F.oxysporum*		*A.flavus*		*A.niger*		*P.chrysogenum*		*C.intermedia*	
	UM4	LM2	UM4	LM2	UM4	LM2	UM4	LM2	UM4	LM2	UM4	LM2	UM4	LM2
S.s (Farm saved)	43.9^{a}	41.2^{a}	35.5^{a}	33.6^{a}	41.0^{a}	40.3^{a}	37.5^{a}	34.2^{a}	31.6^{a}	28.2^{a}	26.8^{a}	23.5^{a}	3.0^{a}	3.2^{a}
S.s (market)	42.4^{a}	41.5^{a}	41.5^{a}	40.2^{a}	46.7^{a}	44.9^{a}	35.5^{a}	31.6^{a}	28.2^{a}	27.1^{a}	29.8^{a}	22.6^{a}	3.1^{a}	3.3^{a}
S.s (certified seed)	6.8^{c}	6.7^{c}	8^{b}	7^{b}	7.5^{c}	6.8^{c}	6.5^{c}	5.8^{c}	2.2^{c}	2.4^{c}	2.5^{c}	1.2^{c}	0.0^{b}	0.5^{b}
S.v (farm saved)	22.5^{b}	21.4^{b}	31.5^{a}	28.8^{a}	28.5^{b}	27.2^{b}	22.5^{b}	18.2^{b}	12.5^{b}	12.2^{b}	12.6^{b}	11.4^{b}	2.5^{a}	2.3^{a}
S.v (market)	23.9^{b}	22.8^{b}	32.5^{a}	31.4^{a}	24.5^{b}	23.4^{b}	23.4^{b}	17.4^{b}	16.7^{b}	16.4^{b}	11.9^{b}	12.8^{b}	2.4^{a}	2.4^{a}
S.v (certified seed)	6.2^{c}	6.3^{c}	8.0^{b}	7.2^{b}	7.0^{c}	6.9^{c}	5.5^{c}	6.2^{c}	2.6^{c}	2.2^{c}	1.2^{c}	1.0^{c}	0.5^{b}	0.6^{b}
Lsd ($P \leq .05$)	11.9	10.8	14.7	12.6	13.9	12.1	12.7	9.8	8.6	7.2	9.7	7.6	1.4	1.5
CV (%)	27.0	26.8	30.4	28.6	28.9	29.2	17.3	22.2	31.5	24.8	24.5	18.9	33.1	28.2

Means followed by the same letter(s) within each column are not significantly different at $P \leq .05$, LSD - Least significant difference at $P \leq .05$, CV- coefficient of variation, Ss-*Solanum scabrum*, Sv-*Solanum villosum*, UM4 - upper midland zone four and LM2-lower midland zone two.

Table 4.4: Frequency (%) of detection of seed-borne fungi in African nightshade seed samples from different locations and seed sources.

Fungal isolate	Method	location	Seed source	Method location	Method x seed source	Rep (Method x Location)	Rep (Method x seed source)
DF	1	3	2	3	2	24	18
Alternaria solani	14.6^{c}	11.4^{b}	18.2^{c}	10.7^{bc}	16.2^{b}	10.4^{b}	10.5^{b}
Fusarium solani	13.6^{bc}	24.1^{c}	26.1^{d}	21.9^{d}	7.4^{a}	10.5^{b}	10.4^{b}
Fusarium oxysporum	15.7^{c}	22.3^{c}	14.6^{bc}	15.2^{c}	14.6^{b}	10.6^{b}	10.2^{b}
Aspergillus niger	24.1^{d}	30.3^{d}	17.7^{c}	23.3^{d}	23.3^{c}	19.2^{c}	16.3^{c}
Aspergillus flavus	26.2^{d}	22.2^{c}	19.4^{c}	15.1^{c}	16.3^{b}	21.8^{c}	20.3^{c}
Penicilliu m Chrysogen umm	9.4^{b}	15.2^{b}	12.4^{b}	8.1^{b}	10.8^{b}	6.5^{ab}	4.4^{a}
Curvularia intermedia	1.3^{a}	2.4^{a}	2.3^{a}	2.0^{a}	2.2^{a}	1.9^{a}	1.8^{a}
LSD	4.6	5.1	3.4	5.6	6.1	7.2	4.2
CV (%)	23.1	21.4	16.8	32.1	19.1	22.6	34.2

Means followed by the same letter (s) within each column are not significantly different at P ≤ .05; LSD - Least significant difference at ≤ .05; CV- coefficient of variation; %-Percentage and Rep-replication

The level of seedling infection differed ($p \leq 0.05$) significantly among the seed sources. Farm saved seed had the highest level of infection followed by seed from local market and the lowest was in certified seed. *Alternaria solani* was the most detected (14.2%) in farm saved seed and the lowest was *Curvularia intermedia* at 1.2%. *Alternaria solani* was the most isolated at 10.3% in market seed and the lowest was *Curvularia intermedia* (1.1%). Certified seed had *Alternaria solani* (3.4%) and *Fusarium solani* (3.5%) being the highest and the lowest was *Curvularia intermedia* (0.5%) (Figure 4.2).

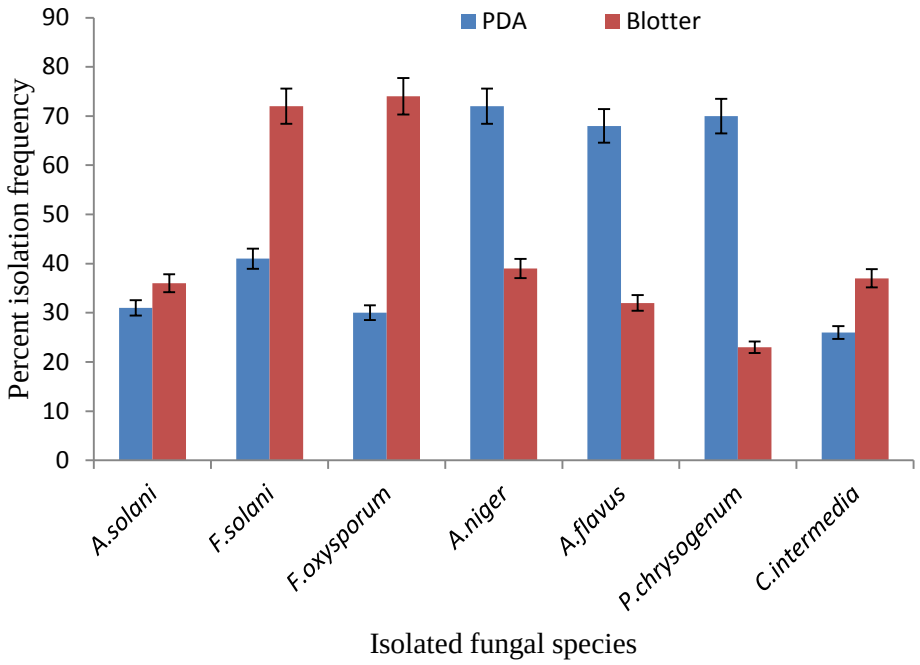


Figure 4.1: Frequency (%) of seed-borne fungi in African nightshade seed samples from different locations. *A: Alternaria, F: Fusarium, A: Aspergillus, P: Penicillium* and *C: Curvularia.* Separation of means at $p \leq 0.05$.

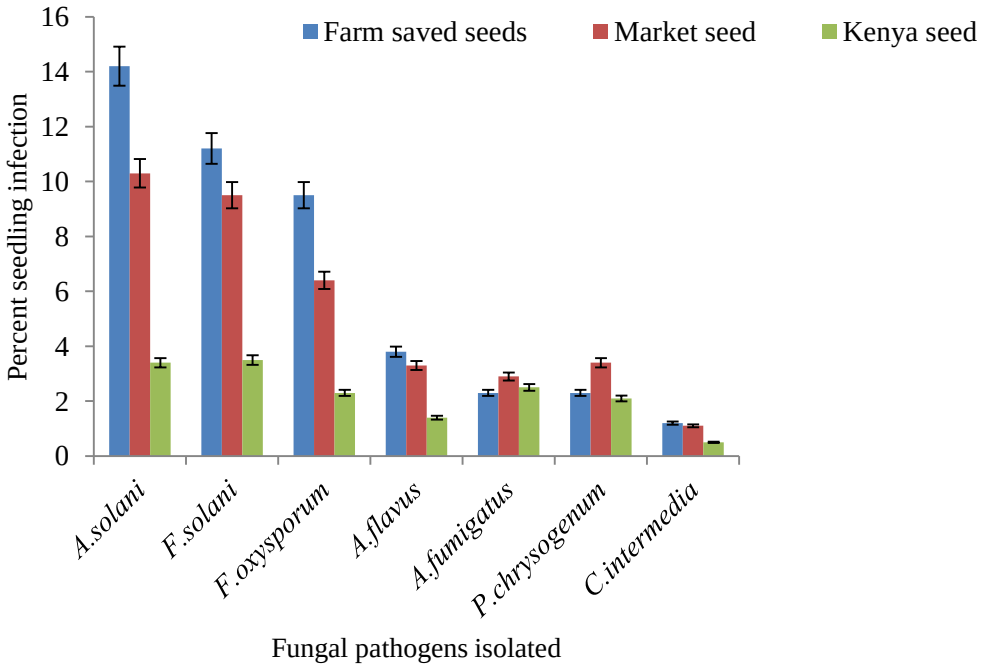


Figure 4.2: Fungal infections on seedling grown under greenhouse conditions from seeds obtained from different sources. Separation of means at $p \leq 0.05$.

4.4.2 Incidence (%) of bacterial pathogens isolated from seed samples of *Solanum scabrum* and *S. villosum*

Xanthomonas campestris pv. *vesicatoria* which causes bacterial leaf spot and *Pseudomonas syringae* pv. *tomato* that causes bacterial speck were isolated from the samples of African nightshade seed (Table 4.5). Biochemical tests revealed that *X. campestris* pv. *vesicatoria* is a gram negative organism and positive for KOH solubility test. It was positive for catalase test, gelatin hydrolysis, starch hydrolysis, arginine dehydrolase, H_2S production and tobacco hypersensitivity reaction and negative on levan test (Table 4.5). *Pseudomonas syringae* pv. *tomato* (brown-black leaf spots) was positive for KOH solubility and the bacterium was gram negative. It was also negative for levan, arginine dehydrolase activity and H_2S production tests. In addition, it was positive for catalase, gelatin hydrolysis and tobacco hypersensitivity reaction (Table 4.5).

Table 4.5: Biochemical characterization of two bacterial pathogens isolated from seeds of *Solanum* spp.

Biochemical test	Bacterial pathogens	
	Xanthomonas campestris pv. *vesicatoria*	*Pseudomonas syringae* pv. *tomato*
Gram's staining	-	-
Catalase test	+	+
Levan formation	-	-
Gelatin hydrolysis	+	+
Starch hydrolysis	+	-
Arginine dehydrolase	+	-
KOH test	+	+
H_2S production	+	-
Tobacco hypersensitivity reaction	+	+

All tests were conducted in 4 replicates and repeated 3 times. '+' indicates +ve reaction; '–' indicates –ve reaction.

The frequency of isolation differed ($p \leq 0.05$) significantly among the agro ecological zones and seed sources. For example, in farm saved seed, *X. campestris* pv. *vesicatoria* was recorded with the highest frequency at 31% in LH2 while *Pseudomonas syringae* had the highest frequency

of 17% in UM4. In seed from local market seed *X. campestris* had an occurrence of 23% which was the highest in UM4 while *P. syringae* pv. *tomato* at 12% was the highest in LH2. A low frequency of *X. campestris* at 2% was recorded in certified seed while *P. syringae* was not detected (Figure 4.3).

Comparing the agro ecological zones, farm saved seed in LH2 had the highest level of *X. campestris* at 31% while the lowest was in UM2 market seed, at 14%. *P. syringae* was highest in farm saved seed UM4 at 17% and lowest from market seed in UM4 (3%) (Figure 4.3). The level of bacterial infection differed ($p \leq 0.05$) significantly in seed from different sources with farm saved seed having the highest frequency of bacterial infection, followed by seed from the local market seed while certified seed had the least bacterial infection. Farm saved seed had the highest level of *Xanthomonas campestris* detected in LH2 at 31% followed by seeds from the local market UM4 (23%) while certified seed had the least at 2%. The same was observed for *Pseudomonas syringae* in farm saved seed being the highest in UM4 at 17% followed by seed from local market in LH2 at 12%, the certified seed had no *P. syringae* detected (Figure 4.3).

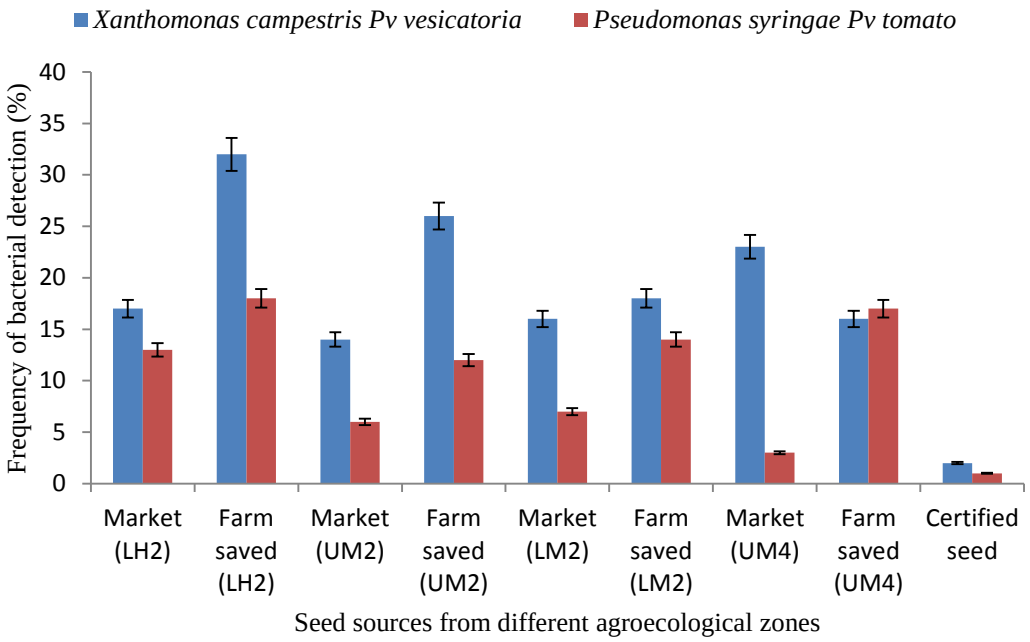


Figure 4.3: Frequency (%) of detection of pathogenic bacterial species isolated from seed sources in different agro ecological zones. UM2-Upper midland zone two; LH2-lower highland zone two; UM4-upper midland zone four; LM2-lower midland zone two. Separation of means at $p \leq 0.05$

4.4.3 Seed to seedling transmission of bacterial pathogens

Seedling grow-out tests showed significant ($p \leq 0.05$) differences in percent seedling infection comparing seed sources and bacterial species. Certified seed had the highest germination percentage of 93% in LH2 while the lowest was in farm saved seed in LM2 and LH2 at 68%. The percentage infection with bacterial pathogens was highest in UM2 at 42% in farm saved seed and the least infection with bacteria was in certified seeds at 9.0% in LM2. *Xanthomonas campestris* pv. *vesicatoria* was detected with the highest infection rates at 43% in UM2 and the lowest in certified seed in LM2 at 7.8%. *Pseudomonas syringae* pv. *tomato* had the highest percent infestation recorded in farm saved seed at 35% in UM4 and the least at 4.8% in UM4 certified seed (Table 4.6).

Table 4.6: Frequency of detection of bacterial pathogens in seedlings, 14 days post germination under greenhouse conditions.

Place of collection (AEZ)	Seed source	Germination (%)	% diseased plants	Percent pathogen infection	
				Xanthomonas campestris pv. *vesicatoria*	*Pseudomonas syringae*. pv. *tomato*
UM2	Farm saved	70[a]	42.0[b]	43.0[b]	31.0[b]
	Market	74[ab]	34.8[b]	38.2[b]	26.4[b]
	certified seed	91[b]	12.8[a]	11.2[a]	8.4[a]
LH2	Farm saved	68[a]	39.0[b]	41.0[b]	28.0[b]
	Market	75[ab]	28.6[b]	34.6[b]	30.2[b]
	certified seed	93[b]	10.9[a]	12.0[a]	6.3[a]
UM4	Farm saved	69[a]	31.0[b]	38.0[b]	35.0[b]
	Market	72[ab]	26.4[b]	32.8[b]	31.6[b]
	certified seed	88[b]	9.8[a]	8.6[a]	4.8[a]
LM2	Farm saved	68[a]	26.8[b]	41.2[b]	33.0[b]
	Market	75[ab]	25.0[b]	38.0[b]	30.0[b]
	certified seed	92[b]	9.0[a]	7.8[a]	5.6[a]
[1]LSD		17.4	14.2	14..2	6.9
[2]CV (%)		27.2	34.5	30.2	28.2

UM2-Upper midland zone two; LH2-lower highland zone two; UM4-upper midland zone four; LM2-lower midland zone two.[1]Least significance difference and [2]Coefficient of variation. Means followed by the same letter(s) within each column are not significantly different at $P \leq .05$.

4.4.4 Field survey results

The leaf samples obtained from the field had a variety of visible symptoms that were associated with virus infection. Some of these symptoms included yellow-green mosaic, stunting, rugosity, vein clearing, yellowing, leaf curling, wilting (Plate 4.1).

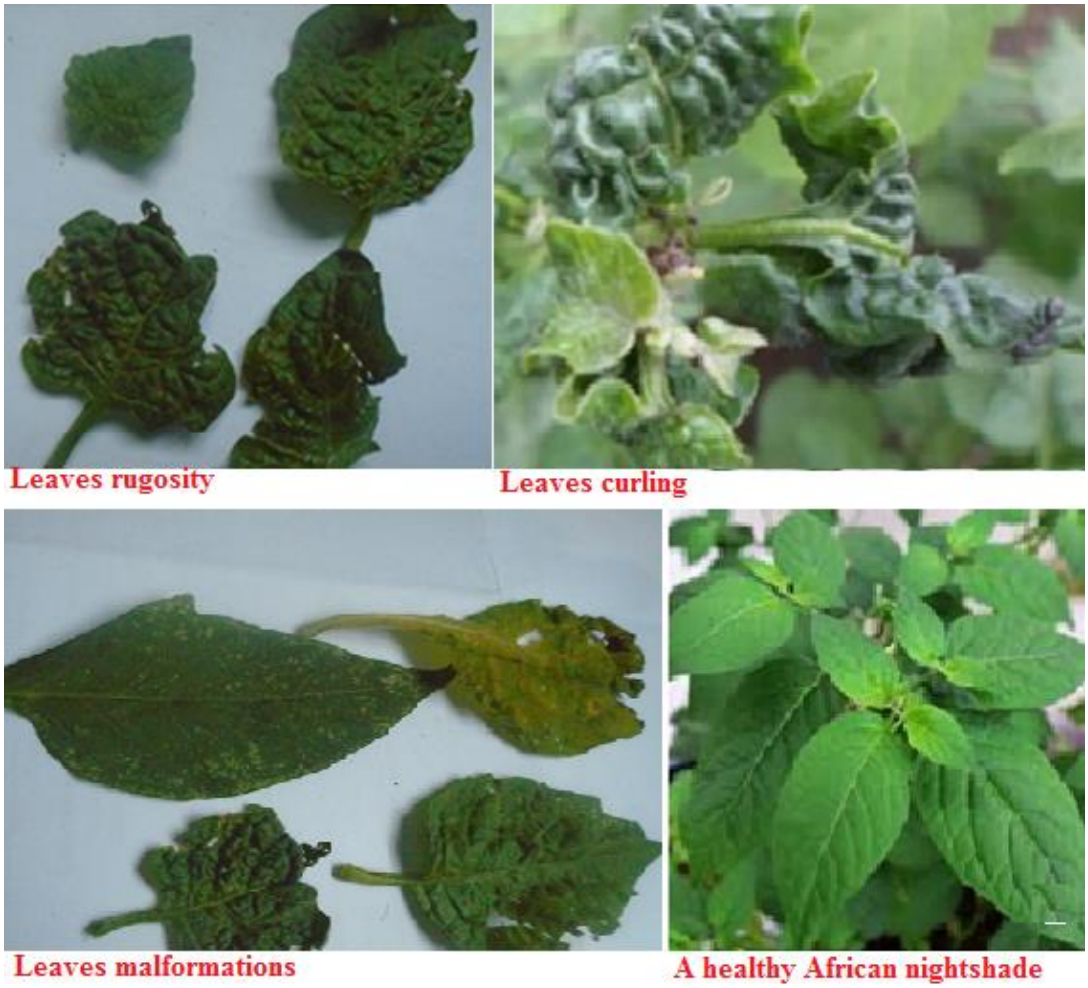


Plate 4.1: Leaf viral like symptoms in African nightshade observed in the field

4.4.5 Serological test

Field and greenhouse samples reacted positively for viruses with ELISA test and had significant ($p \leq 0.05$) differences in percentage frequency for each virus detected as follows: Potyviruses (74%), *Potato virus Y* (PVY) (43%), *Cucumber mosaic virus* (CMV) (38%) and *Tobacco mosaic virus* (TMV) (37%). *Tomato spotted wilt virus* (TSWV) had a percent frequency of 17% in LH2, 8.3% in LM2 and 17% in UM4 for field samples. In addition, greenhouse samples recorded 17% for LH2 while field samples in UM2 and greenhouse; LH2, LM2 and UM4 were negative for TSWV. Certified seed samples reacted negative for virus antigens on ELISA. In addition, all samples reacted negatively for *Tomato yellow leaf curl virus* (TYLCV) and *Pepper mild mottle virus* (PMMoV) (Table 4.6; Figure 4.4).

Table 4.7: Reaction of field and greenhouse samples to virus antigens on ELISA

Virus species	Field samples +ve for Elisa test				Greenhouse samples +ve for Elisa test					X/N (%)
	Suneka (UM2)	Ogembo (LH2)	Lurambi (LM2)	Amalemba (UM4)	Suneka (UM2)	Ogembo (LH2)	Lurambi (LM2)	Amalemba (UM4)	Certified seed	
Potyvirus	23 (96^{a})	22 (92^{a})	22 (92^{a})	21 (88^{a})	21 (88^{a})	20 (83^{a})	19 (79^{a})	13 (54^{b})	0 (0^{a})	74^{a}
Potato virus Y (PVY)	8 (33^{c})	5 (21^{c})	5 (21^{cd})	20 (83^{a})	10 (42^{b})	9 (38^{b})	15 (63^{a})	21 (88^{a})	0 (0^{a})	43^{b}
Cucumber mosaic virus (CMV)	13 (54^{b})	14 (58^{b})	12 (50^{b})	12 (50^{b})	10 (42^{b})	19 (79^{a})	1 (4^{c})	2 (8^{c})	0 (0^{a})	38^{b}
Tobacco mosaic virus (TMV)	23 (96^{a})	10 (42^{bc})	6 (25^{c})	7 (29^{bc})	6 (25^{bc})	15 (63^{a})	9 (43^{b})	3 (13^{c})	0 (0^{a})	37^{b}
Tomato spotted wilt virus (TSWV)	0 (0^{d})	4 (17^{c})	2 (8.3^{cd})	4 (17^{c})	0 (0^{c})	4 (17^{bc})	0 (0^{c})	0 (0^{c})	0 (0^{a})	6.5^{c}
Tomato yellow leaf curl virus (TYLCV)	0 (0^{d})	0 (0^{c})	0 (0^{d})	0 (0^{c})	0 (0^{c})	0 (0^{c})	0 (0^{c})	0 (0^{c})	0 (0^{a})	0^{c}
Pepper mild mottle virus (PMMoV)	0 (0^{d})	0 (0^{c})	0 (0^{d})	0 (0^{c})	0 (0^{c})	0 (0^{c})	0 (0^{c})	0 (0^{c})	0 (0^{a})	0^{c}
Lsd value α=0.05	(19.2)	(23)	(22.9)	(31.2)	(38.2)	(21.7)	(18.2)	(33.7)	(0)	(31.3)

X-number of positive samples-N-total number of samples, numbers in parenthesis indicate percentage incidence. Values with the same letters in columns do not differ significantly at α=0.05 (means in parenthesis). LSD- Least Significant Difference. UM2-Upper midland zone two; LH2-Lower highland zone two; UM4-Upper midland zone four; LM2-lower midland zone two.

4.4.6 Detection of viral pathogens

Field leaf samples had the highest percent incidence of potyviruses (31%), PVY (28%), CMV (23%), TMV (13.6%) and TSWV (6.7%). Seed samples had the second highest percentage frequency of virus detected as follows: potyviruses (PVY, PVS and PVX), (15.2%), PVY (8.9%), CMV (7.3%), TMV (5.6%) and TSWV (1.8%). Greenhouse plant samples had the least percentage frequency of potyviruses (PVY, PVS and PVX) of 11%, PVY (8.2%), CMV (3.3%), TMV (2.3%), TSWV (1%) (Figure 4.4). TYLCV and PMMoV were not detected in all the samples tested.

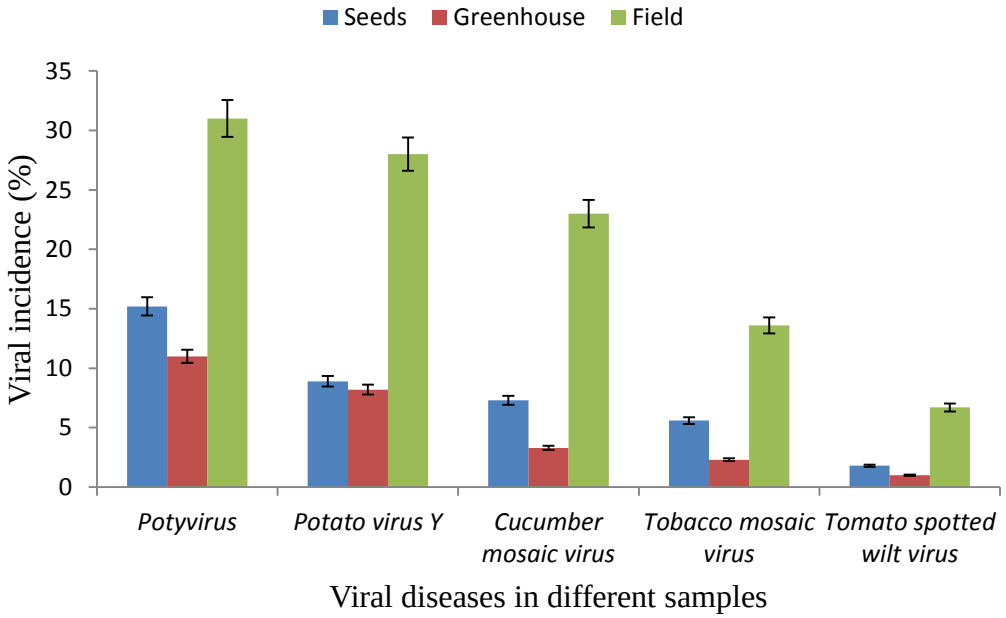


Figure 4.4: Percent viral incidence in seed, field and greenhouse samples. Separation of means at $p \leq 0.05$.

4.4.7 Molecular detection of viruses in African nightshade

Potyviruses were detected in plant samples from field and greenhouse. The results of PCR showed that all samples obtained from the field in the agroecologies LH2 and UM4 reacted positively for potyviruses presence. Half of those from UM2 and UM4 were positive for potyviruses. All greenhouse samples reacted positive. However, certified seeds reacted

negatively for potyvirus. The PCR product sizes for potyviruses were PVY (577bp), PVS (426bp) and PVX (337bp) and *potato virus Y* strains (181bp=$PVY^{N: O}$ (necrotic recombinant), 267bp=PVY^{O} (ordinary) & 452 bp=PVY^{NTN} (necrotic) on 1.2% agarose gel (Plate 4.2 & 4.3).

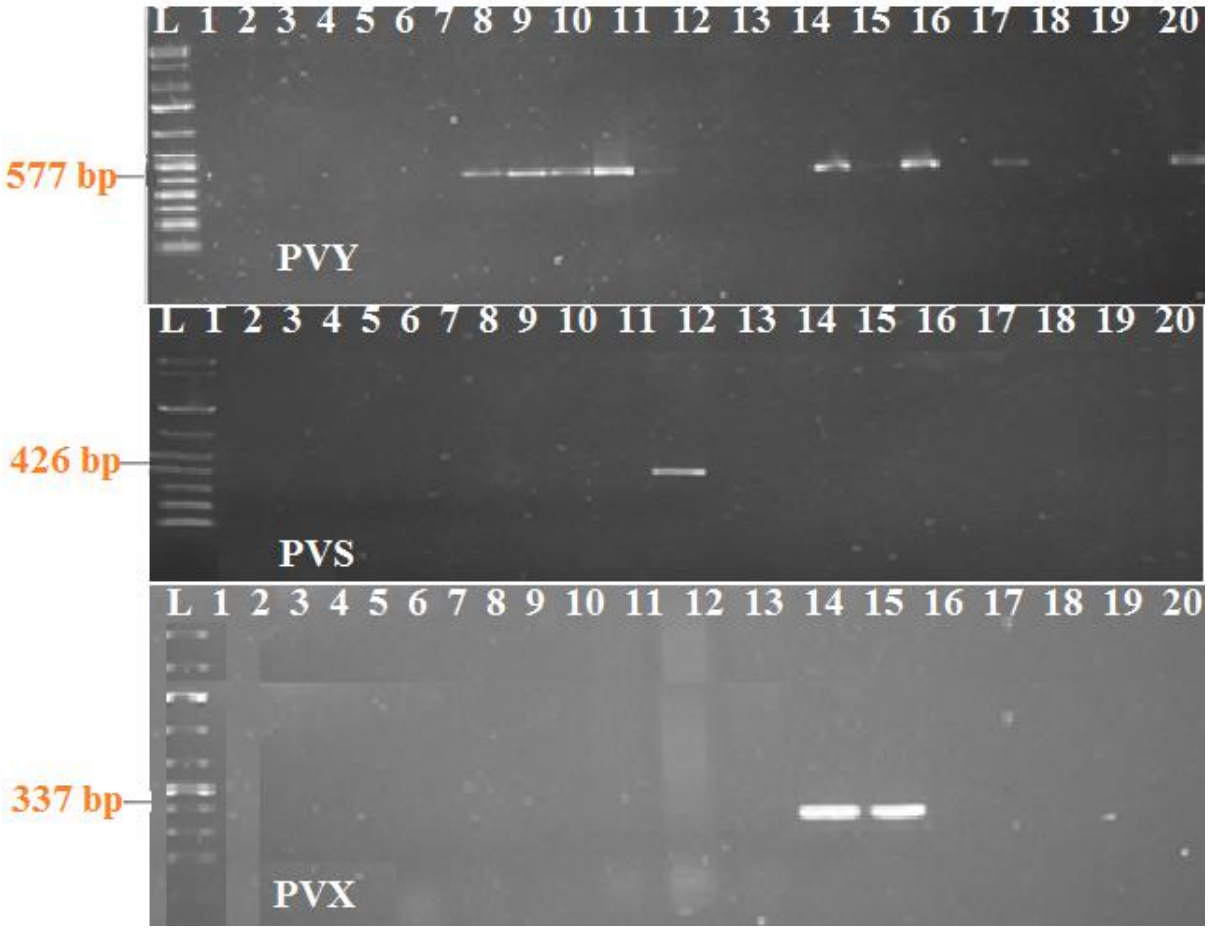


Plate 4.2: Detection of potyviruses in seed, field and greenhouse samples of African nightshade from agro ecological zones (UM2, LH2, UM4 and LM2) using RT-PCR. PVY: seed samples lanes 1-5, field samples lanes 6-13, greenhouse samples lanes 14-18, negative control lane 19 and positive control lane 20. PVS: seed samples lanes 1-5, positive control lane 12, negative control lane 13, field samples lanes 6-11, 14 & 15 and greenhouse samples lanes 16-20. PVX: seed samples lanes 1-5, field samples lanes 6-13, positive control lanes 14 &15 and negative control lane 16 and greenhouse samples lanes 17-20. Potyviruses: *Potato virus Y* (PVY), *Potato virus S* (PVS) and *Potato virus X* (PVX).

Plate 4.3: Detection of *Potato virus Y* strains in African nightshades from tissues obtained from seed, field and greenhouse from different agro ecological zones using RT-PCR. Lane L, 1-kb DNA ladder; lane 1 (positive control); Lane 2&3 (negative control); Lane 4-7: certified seed (UM2, LH2, UM4 & LM2, respectively); Lane 8-11: greenhouse samples (UM2, LH2, UM4 &LM2, respectively); Lane 16-20: field samples (UM2, LH2, UM4 &LM2, respectively) (181bp=$PVY^{N:O}$ (necrotic recombinant), 267bp=PVY^{O} (ordinary) & 452 bp=PVY^{NTN} (necrotic)).

CMV and TMV were detected in field and greenhouse samples (Plate 4.4 & 4.5) while TSWV and TYLCV tested negative on PCR. All field samples from UM2 and UM4 were positive for CMV. Half of the samples from LM2 tested positive while all samples from LH2 tested negative for CMV. In addition, all greenhouse, field and certified seed samples tested negative for CMV. Field samples from agroecologies UM2 and LH2 tested negative for TMV. Fifty percent of UM4 samples tested positive for TMV while, all samples tested positive for TMV in LM2. Fifty percent of greenhouse samples from LH2, LM2 and UM4 tested positive for TMV. The observed PCR product sizes were CMV (200 bp; 593bp) and TMV (512bp) on 1.2% agarose gel following electrophoresis (Plate 4.4 & 4.5).

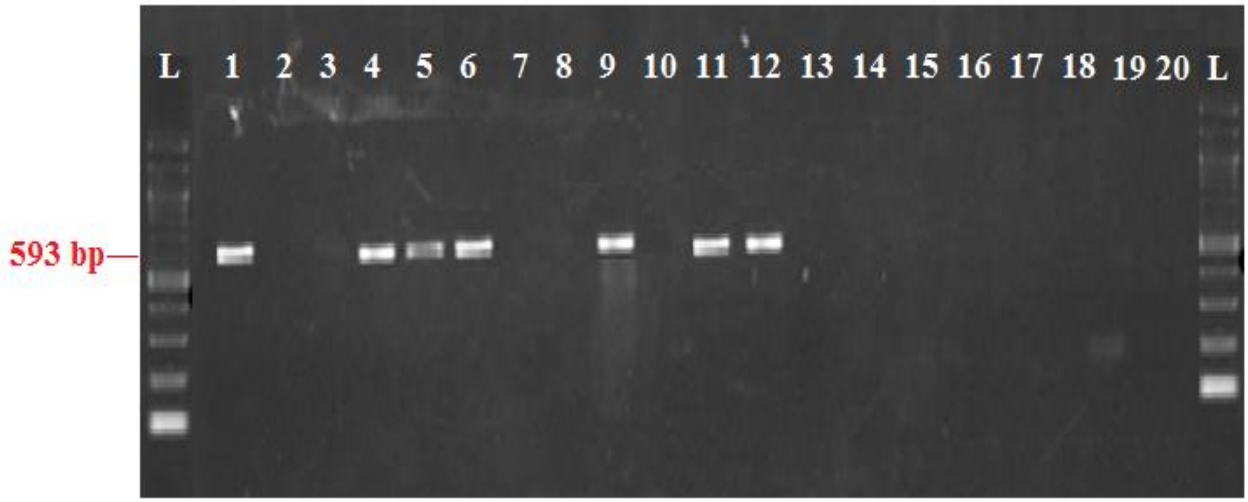


Plate 4.4: Detection of *Cucumber mosaic virus* (CMV-593 bp) in samples of African nightshade leaf tissues using multiplex RT-PCR. Lane 1-positive control, 2 & 3 (uninfected samples; negative control; from certified seed), lane 4, 5 & 6 UM2 field samples, lane 7 & 8 LH2 field samples, lane 9 & 10 LM2 field samples and lane 11&12 UM4 field samples. lane 13 & 14 UM2 greenhouse samples, lane 15 & 16 LH2 greenhouse samples, lane 17&18 LM2 greenhouse samples, lane 19 & 20 UM4 greenhouse samples and lane L; 1-kb DNA ladder.

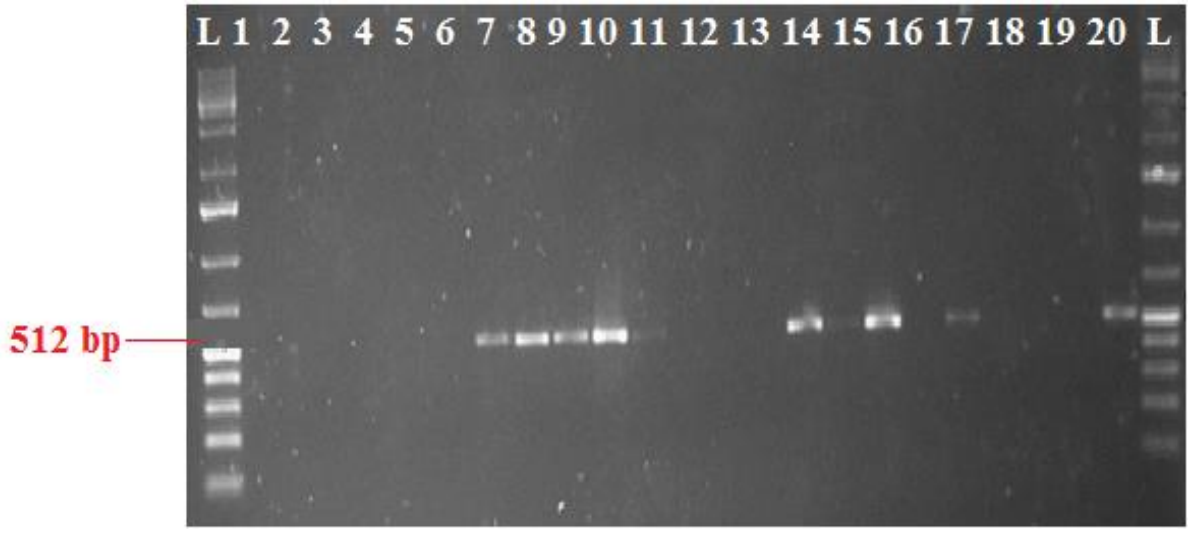


Plate 4.5: Detection of *Tobacco mosaic virus* (TMV-512 bp) in African nightshade leaf tissues using multiplex RT-PCR. Lane L;1-kb DNA ladder, lane 1,2 & 3 UM2 field samples¸ lane 4,5 & 6 LH2 field samples, lane 7,8 & 9 LM2 field samples, lane 10,11 &12 UM4 field samples, lane 13 & 14 UM2 greenhouse samples, lane 15 & 16 LH2 greenhouse samples, lane 17&18 LM2 greenhouse samples, lane 19& 20 UM4 Greenhouse samples, lane 21 & 22 (uninfected samples; negative control; from certified seed) and lane 23-positive control.

4.5 Discussion

A wide range of plant pathogenic fungi, bacteria and viruses were isolated from African nightshade seed and leaf tissues. The fungal pathogens were *Alternaria solani, Fusarium solani, Fusarium oxysporum, Aspergillus niger, Aspergillus flavus, Penicillium chrysogenum* and *Curvularia intermedia.* Bacterial pathogens detected were *Xanthomonas campestris* pv. *vesicatoria* and *Pseudomonas syringae* pv. *tomato.* Potyviruses species detected included; *Potato virus Y* (PVY), *Potato virus S* (PVS) and *Potato virus X* (PVX). In addition, *Cucumber mosaic virus* (CMV), *Tobacco mosaic virus* (TMV) and *Tomato spotted wilt virus* (TSWV) were also detected. Scanty work has been done on viruses infecting African nightshade and this study compared the findings with those reported by Kaur (2010), Lee *et al*. (2011), Cuevas *et al*. (2012), Groves *et al*. (2016) and Hamin *et al*. (2014) on detection of pathogens in tomato and potato.

The present study revealed that farm saved seed and seed sourced from local markets was infected with seed borne pathogens. Earlier studies by Ismael (2010) reported that Aspergillus, Cladosporium and Fusarium genera were the most abundant pathogens associated with tomato seed. Hamin *et al. (*2014) detected *Aspergillus flavus, Aspergillus niger, Curvularia lunata, Fusarium spp*. and *Alternaria solani* in tomato seeds. In addition, Kaur (2010) isolated a number of seed borne fungi belonging to the genera Fusarium, Curvularia, Penicillium, Rhizopus, Colletotrichum and Macrophomina in solanaceous crops. Uma and Weseley (2013) further reported that *Aspergillus niger* and *Aspergillus flavus* were found within the seed tissues of solanaceous crops.

African nightshade was infected by bacterial pathogens *Xanthomonas campestris* pv. *vesicatoria* and *Pseudomonas syringae* pv. *tomato.* These pathogens, in association with fungi,

are a major cause of seed rot affecting seed germination and seed quality. Handiseni *et al*. (2008) reported that bacterial pathogens infect traditional leafy vegetables and the important genera are *Xanthomonas* and *Pseudomonas*. The source of bacteria inoculum on farm saved seed could have been from plant debris as reported by Naseri and Mousari (2015).

Using serology and molecular techniques the study detected viruses in the genera potyviruses, begomoviruses, tobamoviruses, cucumovirus and tospoviruses. The use of symptoms was not adequate in virus detection as demonstrated by use of serology and molecular methods. The use of symptomatology as a traditional method has its shortcomings since some symptoms may be as a result of environmental factors and also not all symptomless plants are negative for viruses due to latent infections (Naidu and Hughes, 2003).

Apart from confirming virus presence in the field samples, this study demonstrated seed transmission of viruses. Studies have shown that seed transmission is the source of primary inoculum in the field but it depends on the survival of embryo during seed maturation. According to Robert *et al*. (2003) seed transmission rates of potyviruses in potato and tomatoes ranged from 2.6 to 30.6%. The viruses invade the cells during the early stages of seed development through transient vesicles present at the suspensor in the micropylar region then enter the seed embryo (Kaur, 2010). In addition, transmission of potyviruses from plant to plant is mainly by aphids as vectors (Lee *et al*.2011). More than 32 species of aphids transmit PVY in a non-persistent manner (Cuevas *et al*. 2012). PVY is responsible for decreases in yield and quality, and just like in potatoes, there is need for strict tolerance limits in seed certification (Abbas *et al*., 2014). Promoting quality production of African nightshades seeds will not only lead to increased yields but also reduce the risk of transmitting the virus by acting as alternative hosts. *Cucumber mosaic virus* (CMV) is generally transmitted by aphid vectors, but the virus

can also be transmitted through seed in some plant species (Ali and Kobayashim, 2010). Transmission of viruses through seed, even at very low rates, is important for virus perpetuation. Results of this study agree with those of Juliane *et al.* (2015) that *Cucumber mosaic virus* and potyviruses infect African indigenous vegetables and can be a challenge to their production. TMV is transmitted through mechanical contact and seed but not vector transmitted. This study has demonstrated TMV transmission through seed, which has been recorded in other crops like tomato and tobacco ranging from 2 to 77% (Wilkinson *et al*., 2006). Transmission of TSWV is mostly associated with thrips and its level of seed transmission is very low (Groves *et al*., 2016). TYLCV and PMMoV reported in other solanaceous crops were not detected in the samples tested.

The high level of pathogen infection in farm saved and market seed could be attributed to poor production, processing and storage of seed. In addition, farmers recycle seeds or borrow from neighbors or buy from market, which is not helpful. Furthermore, there is inadequate certified seed in the market and is also costly. The findings are in agreement with report by Icishahayo (2014) that farm saved seeds are more infected by pathogens because of recycling and poor handling of seed by farmers. The high levels of seed-borne pathogens in farm saved and market seed could have been the major cause of disease transmission to seedlings under greenhouse experiment in this study. In addition, farm saved and market seeds had a lot of inert matter especially from plant debris due to poor processing methods and this could have been the source of infection and cause of poor germination. The findings concur with reports by Ngadze (2014) who reported that pathogens result in poor crop stand due to poor germination or poor quality seedlings which cannot withstand relative environmental changes resulting to dying away of plants. These findings also agree with those of Sabry (2013) who reported high level

of disease transmission in farm saved seed. Moreover, Sharma *et al.* (2008) reported 75% yield losses in crop raised from infected seeds compared to 57% loss from other sources of inoculum.

The low level of fungi, bacteria and viruses on seed sourced from markets compared to farm saved could be due to better sorting and selection of market seeds by traders before selling. Few farmers used certified seed due to cost implications and majority preferred to use farm saved or market seed. This agrees with report by Opole *et al*. (2003) that farmers do not use certified seeds because they are not readily available and are perceived to be expensive compared to farm saved seed. The methods used by seed companies for screening certified seed for infections are not hundred percent effective. In addition, stringent measures to certify seed are not in place for traditional vegetables like other crops hence high level of disease infection.

Fungal diseases were detected by blotter and agar plating method and species identified through microscopy. Blotter method allowed better detection of fungi in the genera *Alternaria, Fusarium* and *Curvularia* while *Aspergillus* and *Pecillium* were better detected by agar plating method. According to Kaur (2010) the blotter method is simple and effective in detecting seed borne pathogens that respond by sporulation while the agar plate method detects seed borne pathogens through colony characteristics. Agar plating, biochemical tests and microscopy were effective in bacterial detection and identification. According to Schaad *et al.* (2001) agar plating and biochemical tests are routinely used for the detection and identification of bacterial pathogens in seeds. The above methods are effective in assessment of the health status of seeds for phytosanitary purposes.

This study found serology and molecular techniques to be effective diagnostic tools for detection of pathogenic viruses. Serological methods (DAS/TAS ELISA) employed in the current study were relatively simple to use, less costly, sensitive, reliable and suitable for testing on a large scale as reported by Abbas (2014). Today, serology testing can be done on ungerminated seeds as was the case in the present study to determine the incidence of virus transmission through seed to seedlings as reported by Sastry (2013).

Reverse transcriptase polymerase chain reaction (RT-PCR) was employed in the current investigation. It was more effective in detecting viruses in the samples because of its specificity, sensitivity and robustness. According to Hull (2009) PCR has the ability to detect low levels of target pathogens and isolate several of them simultaneously. It also enables easy quantification of pathogens on seeds and interpretation of results due to its ability to distinguish between closely related organisms. However, use of serology in assessment of sanitary status is faster, simple and inexpensive.

Pathogen incidence in the four agro ecological zones varied and this could be due to different levels of inocula in the seeds in different farmlands. Infection is favored by the climatic conditions prevailing, handling and presence of other hosts plants which increases the pathogen inoculum. The low infection on *Solanum villosum* compared to *Solanum scabrum* could be due to some level of tolerance of the plant to diseases (Chowdhury *et al.* 2008). The source of resistance is attributed to its bitter taste, antimicrobial and larvicidal properties due to presence of some phenolic compounds that are antagonistic to bacterial and fungal pathogens (Chowdhury *et al.* 2008).

CHAPTER FIVE

IDENTIFICATION OF COMMON VIRUSES INFECTING AFRICAN NIGHTSHADE USING MOLECULAR TECHNIQUES

5.1 Abstract

Plant pathogenic viruses lead to high yield losses and early detection remains a critical factor in virus disease management by preventing introduction and spread into new areas. African nightshade (ANS) is used as a leafy vegetable crop in households and its' demand has continued to increase. In this study, next generation sequencing was utilized for genomic examination of three major viruses infecting ANS obtained from greenhouse and farm fields. The greenhouse samples were raised at the University of Nairobi Research Farm in Kabete and field samples collected from different agroecological zones in Kisii and Kakamega. Leaves of African nightshade exhibiting viral disease symptoms were used to confirm the presence of *Potato virus Y* (PVY), *Cucumber mosaic virus* (CMV) and *Tobacco mosaic virus* (TMV) by Reverse transcriptase polymerase chain reaction (RT-PCR). Sequencing was done using illumina MiSeq platform and three strains of *Potato virus Y* (PVY^{NTN}, PVY^{O} and $PVY^{N:O}$) were identified. The isolates revealed similar genomic identity of greater than 90% with 22 isolates from different parts of the world. *Cucumber mosaic virus* (CMV) was closely related to ten isolates, highly identical above 90% to two Kenyan isolates and those from Asian countries. Ten isolates of CMV clustered in subgroup I while only four were in subgroup II. *Tobacco mosaic virus* (TMV) was homologous above 90% to ten isolates and those of European origin. Detection of these viruses in ANS suggests that they are prevalent in the sampled regions.

5.2 Introduction

The African nightshade (ANS) is attractive to small scale farmers due to minimal financial input requirements. The risks of financial losses are low compared to the exotic vegetables (Mumbi *et al.,* 2006). These vegetables have high micronutrient content, medicinal properties, several agronomic advantages and economic value (AICAD, 2003). The production and yield of ANS is seriously affected by the invasion of emerging and recurrent plant viruses inducing symptoms such as veinal necrosis, mosaic, mottling, yellowing, deformation, shoestring, ringspots and stunting. The use of next generation sequencing (NGS) helped to determine the causal pathogen and the phylogenetic relationships between isolates of the same species (Kerlan *et al*., 2011). PVY exists as a complex of strains (PVY^{NTN}, PVY^{O} and $PVY^{N:O}$) that can be distinguished on the basis of their biology, serology and genome analysis.

PVY has a worldwide distribution with a large host range in solanaceous species including potato, tobacco, tomato, nightshades, petunia and weeds (Singh *et al.*, 2008). CMV is distributed worldwide, can infect more than 1,200 plant species including vegetables, fruit crops, ornamentals and weeds (Wang *et al*.,2011). Cucumoviruses are aphid vectored and also transmitted through contact between plants (Spence *et al*.2001). *Cucumber mosaic virus* (CMV) strains are divided into subgroup I and subgroup II with subgroup I strain further divided into the A and B. *Tobacco mosaic virus* (TMV) belongs to the Genus Tobamoviruses and family Virgaviridae (Adams *et al*.,2005). Tobamoviruses are transmitted through mechanical contact and seed to a significant degree but not by insect vectors. The current study aimed at characterizing the major viruses infecting ANS in Kenya by use of next generation sequencing.

5.3 Materials and methods

5.3.1 Source of virus isolates

Twelve four leaf tissues positive for viruses were used in this study. Three major plant viruses that were earlier detected and reported in the samples that is *Potato virus Y* (PVY), *Cucumber mosaic virus* (CMV) and *Tobacco mosaic virus* (TMV) were retested. The procedures for extraction, isolation and detection of viruses were done as described in sections 4.3.6, 4.3.7 and 4.3.8.

5.3.2 Research sites

Samples were obtained from the greenhouse in crop protection field station at the University of Nairobi and field samples from the agro-ecological zones as described in section 4.3.1. The molecular work was done at the Kenya Plant Health Inspectorate Service (KEPHIS) Muguga quarantine station and Inqaba Biotechnology and Genomics Company (SA).

5.3.3 Next-generation sequencing (NGS)

Twenty four seed and leave samples were selected from 120 samples (described in section 4.3.6) representing the four agroecological zones. Two samples from each of the four agroecological zones used were from seed sources (8), greenhouse (8) and field plant leaves (8). Each sample source represented a pool and the agroecological zones formed the strata. The library sizes were determined, quantified and normalized to ensure even read distribution of samples. Twelve lanes obtained from three pools for each of the four strata and two lanes one for positive and another for healthy control were used for next generation sequencing (NGS). The 14 samples for NGS were validated using reverse transcriptase polymerase chain reaction (RT-PCR). Primers used were as described in Table 4.1 in section 4.3.8. Total RNA extraction and RT-PCR for *Potato virus Y* (PVY), *Cucumber mosaic virus* (CMV) and *Tobacco mosaic virus* (TMV) were done as described in section 4.3.8. Fourteen purified PCR products each

15µl per tube and 12 primers each 10µl per tube were prepared. Ribosomal RNA was depleted with the kit ribo-Zero rRNA removal kit (Illumina, USA) and the RNA integrity number was measured in a 2200 bioanalyzer (Agilent Technologies, USA). The cDNA library was constructed with the TrueSeq RNA sample preparation kit (Illumina, USA) and Next Generation Sequencing (NGS) performed in Illumina HiSeQ 2010 equipment at Inqaba Biotechnology and Genomics Company (South Africa).

5.3.4 Bioinformatics analysis

After Next generation sequencing (NGS), low quality bases were removed from the data set using seqTK (https://github.com). Sequences from PVY, CMV and TMV were identified using BLASTN and BLASTX (http://blast.ncbi.nlm.nih.gov/Blast.cgi) against a local database containing all viruses infecting plants in the ninth report of the International Committee of Taxonomy of viruses (Kings *et al*., 2012). The nucleotide sequences of the gene were aligned with those of corresponding viruses deposited in Genbank by using Clustal-LC software. Sequence homology analyses of the gene were performed using BioEdit version 7 software (http://www.mbio.ncsu.edu/BioEdit.html). Viral genomes were assembled to reference genomes (http://bowtie-bio.sourceforge.net). Assemblies were verified for inconsistences and sequencing errors with Tablet (https://ics.hutton.ac.uk/tablet). Phylogenetic tree was constructed using MEGA 7.0 software with the neighbor-joining algorithm and 1000 bootstrap replications (Kings *et al*., 2012). Consensus sequences for PVY, CMV and TMV were submitted to GenBank to obtain Accession numbers.

5.4 Results

5.4.1 Detection of *Potato virus Y* in African nightshade

Greenhouse and farm field sample were positive for the three virus strains ($PVY^{N:O}$, PVY^{O} and PVY^{NTN}). The positive samples were PVY 1A, 2A, 2B, 3A, 4A and 4B. The negative samples were 1B and 3B. UM2 and UM4 AEZs had one sample each positive for PVY while the another sample was negative while all the greenhouse samples were positive. In addition, all samples from AEZ LH2 and LM2 were positive for PVY. The virus strain with the highest frequency of occurrence was $PVY^{N:O}$ (necrotic recombinant) occurring in 75% of the samples followed by PVY^{O} (ordinary) occurring in 62.5% of the samples and the lowest was PVY^{NTN} (necrotic) occurring in 12.5% of the samples (Plate 5.1).

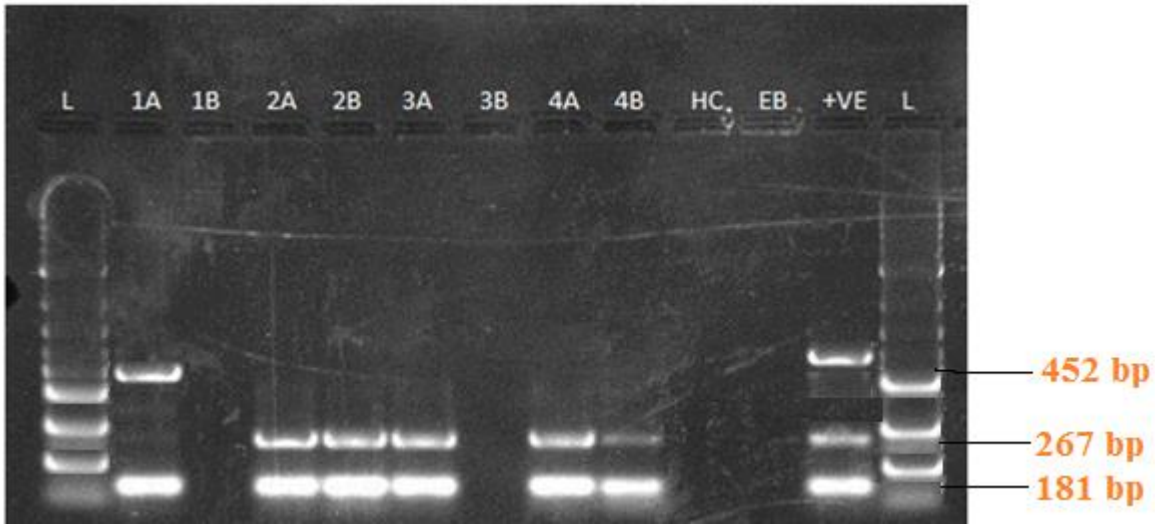


Plate 5.1: Detection of *Potato virus Y* strains in samples of African nightshade from different agro ecological zones using RT-PCR. L-DNA ladder, samples 1A & 1B (UM2), samples 2A & 2B (LH2), samples 3A & 3B (UM4) and sample 4A (LM2). Sample 4B (Greenhouse sample), HC-Healthy Control, EB-Extraction Buffer (blank) and +VE positive control. 181bp=$PVY^{N:O}$ (necrotic recombinant), 267bp=PVY^{O} (ordinary) & 452 bp=PVY^{NTN} (necrotic).

5.4.2 Phylogenetic analysis of *Potato virus Y* in African nightshade

The nucleotide sequences were aligned and used to determine the phylogenetic relationship between isolates and strains. The sequences were deposited in NCBI databank with accession numbers MK905410, MK905411 and MK905412 (Appendix 1). The phylogenetic analysis of PVY in the current study revealed three strains of *Potato virus Y* (PVY^{NTN}, PVY^{O} and $PVY^{N:O}$). The PVY^{NTN} strains clustered with PVY isolates MH603863.1 (South Korea), JN936439.1 (South Africa), MF176827.1 (Colombia), MF440322.1 (Italy), JX43299.1 (Finland) and MF871640.1 (Algeria). PVY^{O} strain clustered with PVY isolates MF624284.1 (USA), KY863548.1 (Egypt) and MF6242287.1 (USA). $PVY^{N:O}$ strain clustered with PVY isolates KY847959.1 (USA), KX356070.1 (Poland) and KY112747.1 from France (Figure 5.1 and Table 5.1). The African nightshades isolates from Kenya had a close nucleotide identity of above 90% with all the isolates as indicated in the phylogenetic tree (Figure 5.1). Comparing PVY isolates for different crops, the African nightshade isolates had a sequence similarity of 84% with those of potato (*Solanum tuberosum*) (Figure 5.1).

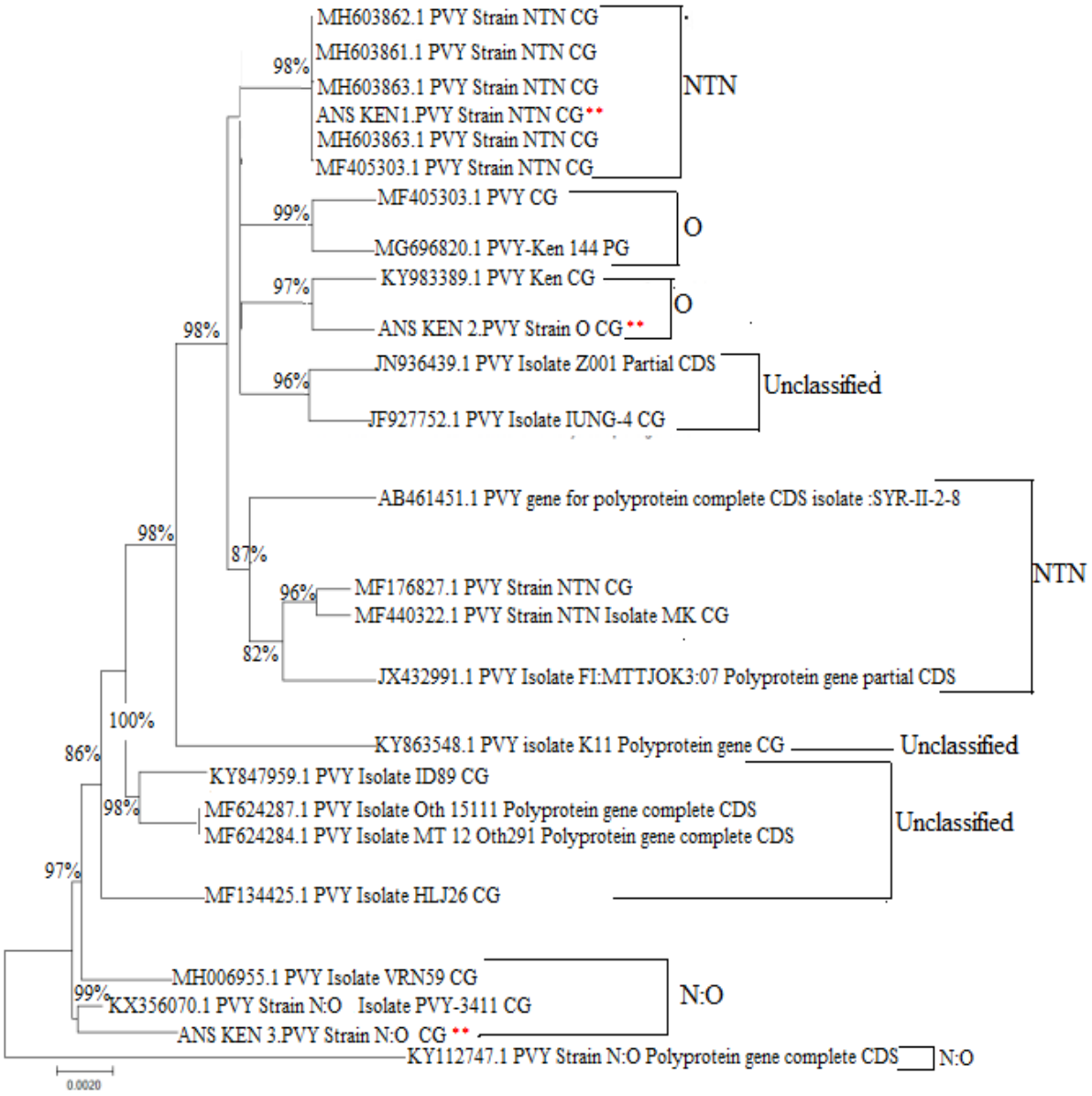


Figure 5.1: Phylogenetic analysis of *Potato virus Y* (PVY) isolates and selected strains, with aligned nucleotide sequences, generated using the neighbor-joining method and MEGA6 software. The percentage of associated taxa clusters in the bootstrap test is shown next to the branches. Strains and serotypes; NTN, N, O, N: O and unclassified PVY are indicated. The tree of the potyvirus was rooted using *Potato virus Y* strain N: O as an out group sequence for phylogenetic analysis. CG-complete genome, PG-partial genome and CDS-CoDing Sequence.

Table 5.1: Similarity of *Potato virus Y* to aligned isolate sequences deposited in the National Center for Biotechnology Information (NCBI) database

Library source	Accession number	Mapped organism	Type	Size (bp)	Strain	Serotype	Host Plant	Phylogenetic identity (%)
Tunisia	MG696820.1	PVY	ssRNA (+ve strand virus)	9657	-	-	*Solanum tuberosum*	99
USA	MF624284.1	PVY	ssRNA (+ve strand virus)	9628	O	O,N-wi	*Solanum tuberosum*	99
South Korea	MH603863.1	PVY	ssRNA (+ve strand virus)	9586	NTN	N	*Solanum tuberosum*	98
South Africa	JN936439.1	PVY	ssRNA (+ve strand virus)	8801	NTN	N	*Solanum tuberosum*	98
Poland	JF927752	PVY	ssRNA (+ve strand virus)	9701	-	-	*Nicotiana tabacum*	98
Colombia	MF176827.1	PVY	ssRNA (+ve strand virus)	9691	NTN	N	*Solanum tuberosum*	98
Italy	MF440322.1	PVY	ssRNA (+ve strand virus)	9702	NTN	N	*Solanum tuberosum*	99
China	KC296437.1	PVY	ssRNA (+ve strand virus)	9703	-	-	*Nicotiana tabacum*	99
Algeria	MF871640.1	PVY	ssRNA (+ve strand virus)	9511	NTN	N	*Solanum tuberosum*	99
Germany	KY848023.1	PVY	ssRNA (+ve strand virus)	9627	O	O	*Solanum tuberosum*	100
China	KY983389.1	PVY	ssRNA (+ve strand virus)	9733	-	-	*Solanum tuberosum*	100
Egypt	KY863548.1	PVY	ssRNA (+ve strand virus)	9626	O	O	*Solanum tuberosum*	99
USA	KY847959.1	PVY	ssRNA (+ve strand virus)	9636	O	O	*Solanum tuberosum*	99
USA	MF624287.1	PVY	ssRNA (+ve strand virus)	9603	O	O	*Solanum tuberosum*	99
China	MF134425.1	PVY	ssRNA (+ve strand virus)	9724	-	-	*Solanum tuberosum*	98
Israel	MH006955.1	PVY	ssRNA (+ve strand virus)	9595	-	-	*Solanum tuberosum*	98
Poland	KX356070.1	PVY	ssRNA (+ve strand virus)	9642	N-wi	O	*Solanum tuberosum*	99

France	KY112747.1	PVY	ssRNA (+ve strand virus)	9636	N:O	O	*Nicotiana tabacum*	100

PVY: *Potato Virus Y*-PVY$^{N:O}$ (necrotic recombinant), PVY^{O} (ordinary) & PVY^{NTN} (necrotic), ssRNA-Single Stranded Ribonucleic Acid.

5.4.3 Detection of *Cucumber mosaic virus* in African nightshade

The following samples were positive for *Cucumber mosaic virus* (CMV) 1A, 3A, 3B, 5A, 6B while samples 1B, 2A, 4A, 4B, 5B and 6A were negative (Plate 5.2). Comparing the agro ecological zones, it's only the Upper midland zone 4 (UM4) which had 100% of the samples positive. Ecological zones UM2, LH2 and UM4 had 50% of the samples positive for *Cucumber mosaic virus* (Plate 5.2).

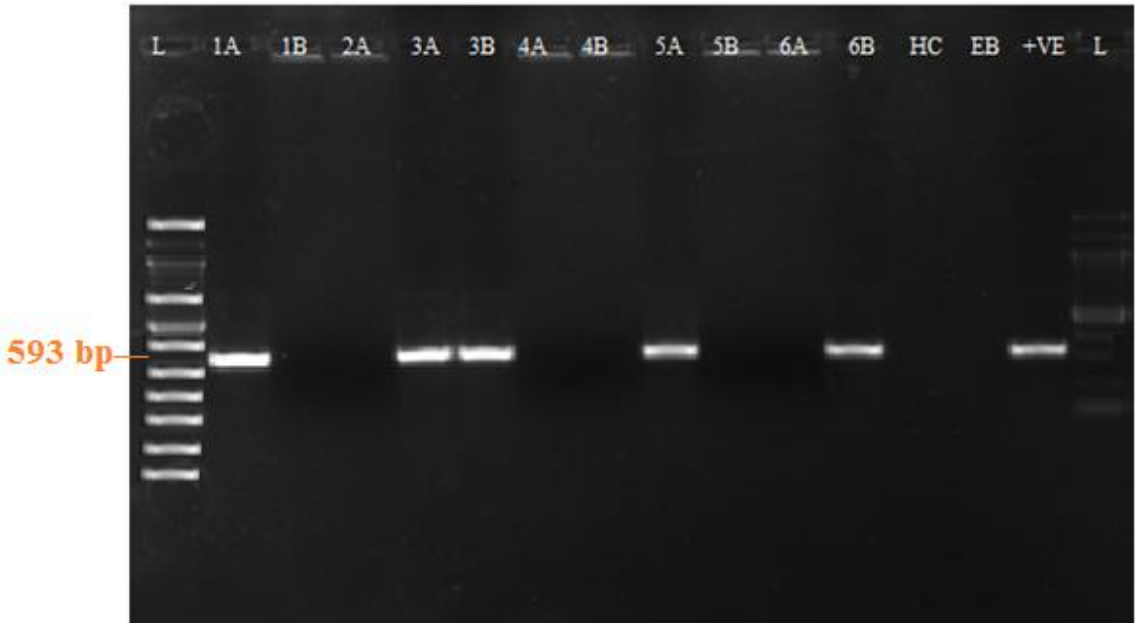


Plate 5.2: Detection of *Cucumber mosaic virus* in samples of African nightshade from farm fields in different Agro ecological zones using RT-PCR. L-DNA ladder, samples 1A & 1B (UM2), samples 2A & 2B (LH2), samples 3A & 3B (UM4), samples 4A & 4B (LM2) and samples 5A, 5B, 6A & 6B

(Greenhouse). HC-Healthy Control, EB-Extraction Buffer (blank) and +VE positive control. 593bp-=CMV.

5.4.4 Phylogenetic analysis of *Cucumber mosaic virus* in African nightshade

Cucumber mosaic virus (CMV) was found in samples from two agroecological zones UM2 and UM4 and absent in LH2 and LM2. Greenhouse samples were positive for the CMV (Plate 5.2). The strains were subdivided into two phylogenetic subgroups I and II (Figure 5.2). The Kenyan isolates shared a genetic similarity of 86 to 100% with isolates from other parts of the world (Figure 5.2). The Kenyan isolates were similar to 10 isolates belonging to subgroup I and 3 isolates in subgroup II (Figure 5.2). The isolates were closely similar (99%) to two CMV isolates obtained from Kenya with accession numbers (MH567342.1 and MH567352.1). The ANS isolates obtained in this study had a similarity of 96% with MGO25947.1 from China, KJ400002.1 and KMO47509.1 from South Korea. In addition, it had 89% similarity to AYA429434.1 from China and AJ580953.1 from Hungary clustering in Subgroup I. Isolates L066456, KC527788 and KP137860 had 87% similarity and clustered in subgroup II. Finally, it had 86% similarity to CMV KC527788 from South Korea and KP137860 from Brazil (Figure 5.2).

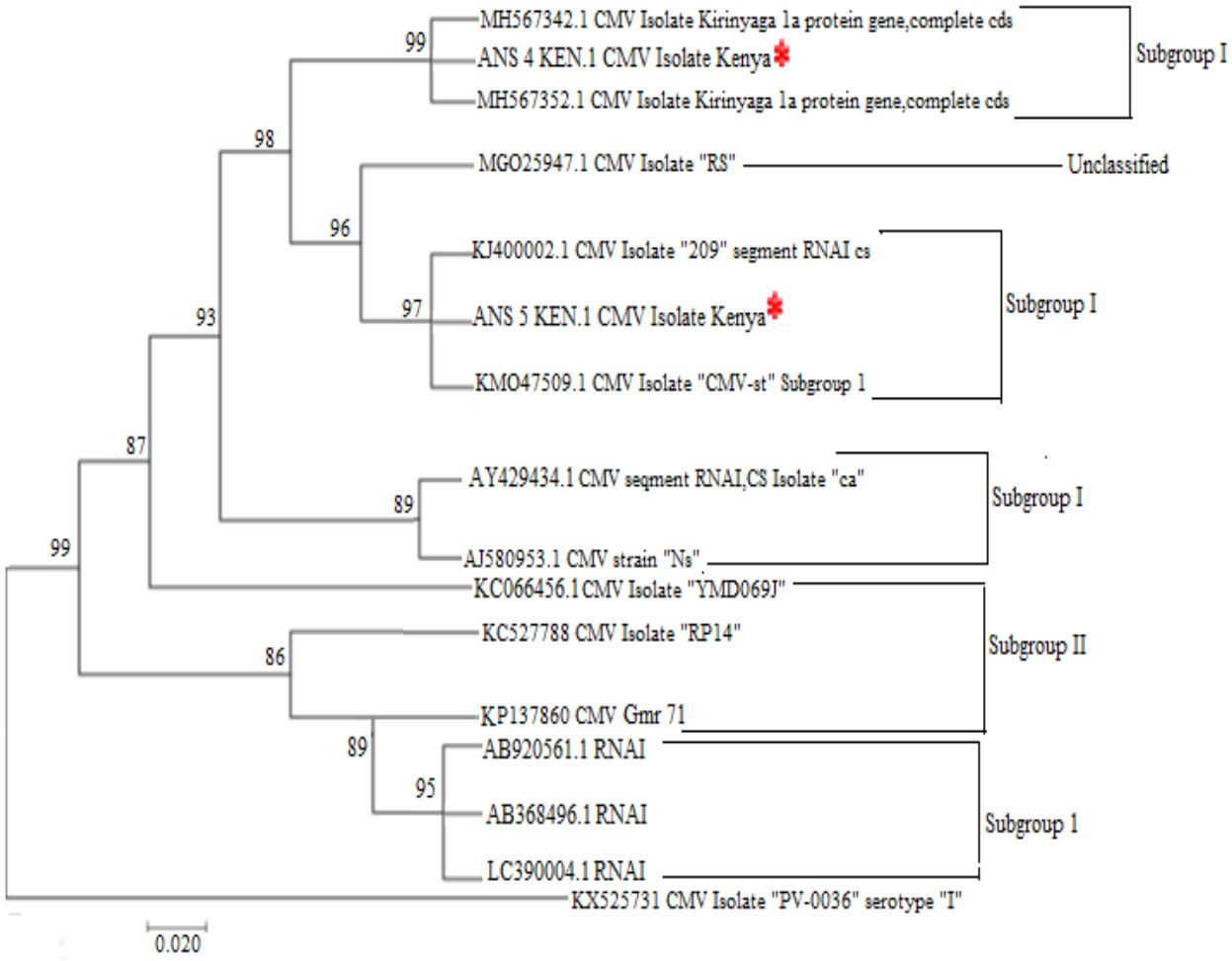


Figure 5.2: Phylogenetic analysis of *Cucumber mosaic virus* (CMV) isolates, with aligned nucleotide sequences, generated using the neighbor-joining method and MEGA6 software. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The tree was rooted using KX525731 CMV Isolate "PV-0036" serotype "I" as an out group sequence for the phylogenetic analysis.

Table 5.2 Sequences in the NCBI database similar to CMV aligned homologous sequences

Library source	Accession number	Host plant species	Mapped organism	Size (bp)	% identify	Segment Description
Kenya-Kirinyaga	MH567342.1	*Phaseolus vulgaris*	CMV	3193	99	RNA 1 1a protein gene
Kenya-Kirinyaga	MH567347.1	*Phaseolus vulgaris*	CMV	3187	99	RNA 1 1a protein gene
South Korea	KJ400002.1	*Glycine soja*	CMV	3191	97	RNA 1 1a protein gene
Brazil	KP137860.1	*Glycine max*	CMV	3144	86	Gmr71
China	MG025947.1	*Solanum tuberosum*	CMV	3309	97	RNA 1 1a protein gene
South Korea	KM047509.1	*Solanum tuberosum*	CMV	3370	97	RNA 1 1a protein gene
Hungary	AJ580953.1	*Nicotiana glutinosa*	CMV	3366	89	RNA 1 1a protein gene
China	AY429435.1	*Arachis hypogea*	CMV	3356	89	RNA 1 1a protein gene
Japan	KC066456.1	*Raphanus sativus*	CMV	3355	87	RNA 1 1a protein gene
South Korea	KC527788.1	*Capsicum annum*	CMV	3360	86	RNA 1 1a protein gene
Germany	KX525731	*Nicotiana benthamiana*	CMV	3369	99	RNA 1 1a protein gene
Japan	AB920561.1	*Nicotiana tabacum*	CMV	3370	95	RNA 1 1a protein gene
Japan	AB368496.1	*Cucumis sativus*	CMV	3371	95	RNA 1 1a protein gene
South Korea	LC390004.1	*Zinnia elegans*	CMV	3358	95	RNA 1 1a protein gene

5.4.5 Detection of *Tobacco mosaic virus* in African nightshade

All samples from agroecological zones UM4, LH2 and LM2 tested positive for *Tobacco mosaic virus* (TMV) except those from UM2 which were negative for the virus using reverse transcriptase chain reaction (Plate 5.3).

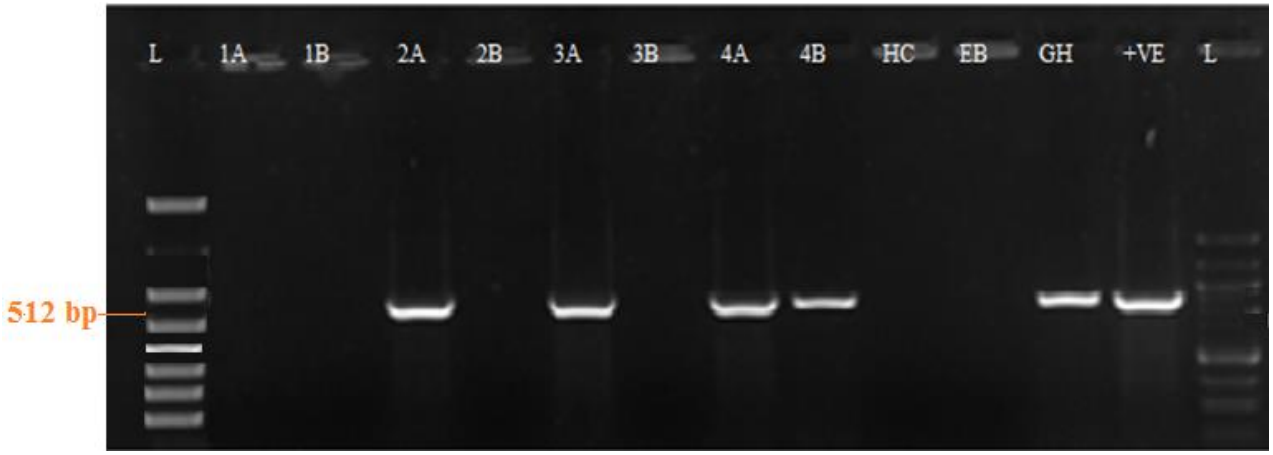


Plate 5.3: Detection of *Tobacco mosaic virus* in samples of African nightshade from farm field and greenhouse of different agro ecological zones using RT-PCR.L-DNA ladder, samples 1A & 1B (UM2), samples 2A & 2B (LH2), samples 3A & 3B (UM4) and samples 4A & 4B (LM2). GH-Greenhouse, HC-Healthy Control, EB-Extraction Buffer (blank) and +VE positive control. 512bp=TMV.

5.4.6 Phylogenetic analysis of *Tobacco mosaic virus* in African nightshade

Tobacco mosaic virus (TMV) sequence blasted in NCBI revealed greater than 90% homology with ten TMV isolates. TMV KY810785.1 strain FERA 111011 from Britain formed clade 1 with TMV AF273221.1 from USA and the Kenyan ANS isolate with 97% identity. TMV KF972435.1 TMV isolate "Tor2-L2" from Spain, AF546184.1 from Finland and KF972436.1 from Spain formed clade II and were 96% homologous with Kenyan ANS isolate. In addition, TMV MG763753.1 isolate Hz from China is clustered with isolates TMV JQ895560.1 "TMV-Soyin" from India and TMV HE818428.1 Hongta-1 from China with 95% homology and formed clade 3. Finally, TMV KT923121.1 *Pepper mild mottle virus* from Brazil was used as an outgroup sequence (Figure 5.3).

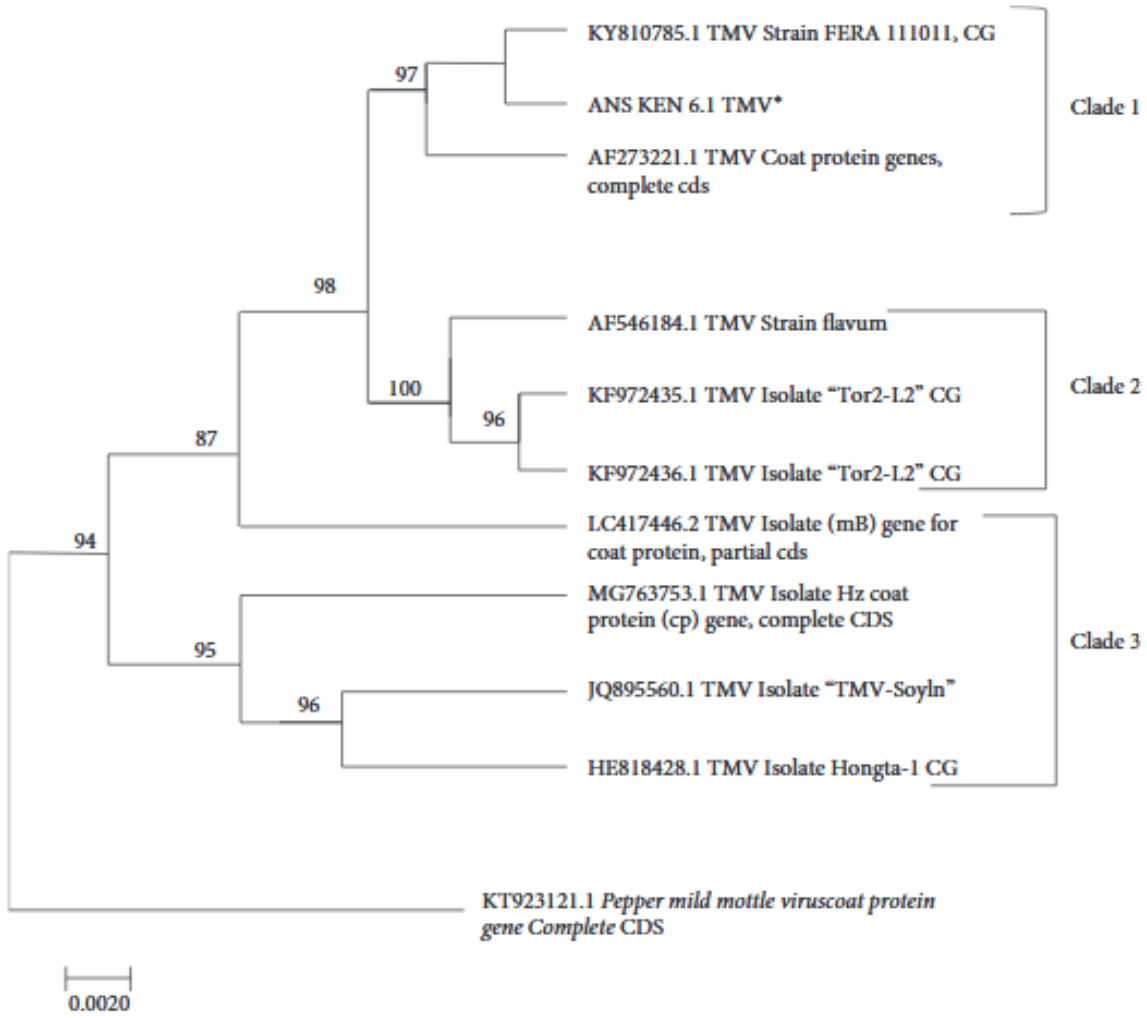


Figure 5.3: Phylogenetic analysis of *Tobacco mosaic virus* (TMV) isolates, with aligned nucleotide sequences, generated using the neighbor-joining method and MEGA6 software. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The tree was rooted using *pepper mild mottle virus* (PMMoV) as an out group sequence for the phylogenetic analysis.

Table 5.3 Sequences in the NCBI database similar to TMV aligned homologous sequences

Library source	Accession number	Host plant species	Mapped organism	Size (bp)	% identify	Segment Description
United Kingdom	KY810785.1	*Nicotiana tabacum*	TMV	6396	97	Complete genome
Germany	AJ429079.1	*Nicotiana tabacum*	TMV	6052	97	Complete genome
USA	AF273221.1	*Nicotiana tabacum*	TMV	6395	97	Complete genome
Finland	AF546184.1	*Nicotiana tabacum*	TMV	1289	99	Isolate flavum Complete genome
Spain	KF972435.1	*Nicotiana benthamiana*	TMV	6276	96	Isolate Tor2 Complete genome
Spain	KF972436.1	*Nicotiana benthamiana*	TMV	6276	96	Isolate ancestor complete genome
Brazil	KT923121.1	*Capsicum annum*	TMV	804	94	Coat protein gene complete cds
India	JQ895560.1	*Nicotiana benthamiana*	TMV	1494	96	Isolate soyln-capsid protein genes Ccds
South Korea	LC417446.2	*Hosta longipes*	TMV	436	87	MB gene partial Cds
China	MG763753.1	*Nicotiana benthamiana*	TMV	480	95	Isolate HZ coat protein
China	HE818428.1	*Nicotiana tabacum*	TMV	6395	96	Isolate Hongta-I complete genome

5.5 Discussion

This study has demonstrated that African nightshade is a host to *Potato virus Y* (PVY), *Cucumber mosaic virus* (CMV) and *Tobacco mosaic virus* (TMV). These viruses were detected in symptomatic African nightshade leaves obtained from greenhouse and farm fields in Nyanza and Western Kenya using RT-PCR and Next Generation Sequencing (NGS). Although data on the pathogenic potential of the viruses is not readily available, intensive cultivation of the crop might lead to build up of inoculum to levels beyond the economic threshold. The detection of these viruses concurs with studies done on other solanaceous crops like potato, tomato and tobacco (Coutts and Jones, 2015). Phylogenetic analysis with Kenyan PVY isolates revealed a similarity homology of more than 90%. Kenyan isolates had a high similarity with 22 isolates from different parts of the world recording a high homology with European strains.

This study revealed that PVY in Kenyan isolates exists as a complex of strains $PVY^{N:O}$ (necrotic recombinant), PVY^{NTN} (necrotic) and PVY^{O} (ordinary) in African nightshade. PVY is considered one of the most dangerous plant virus with different strains causing 80% of plant losses (Tian *et al*., 2011). This study utilized next generation sequencing (NGS) due to its ability to detect viruses in complex infections (Roossink *et al*., 2015). However, it depends on the infecting strains, time of infection and co-infecting species. Similar findings on PVY infections have been reported elsewhere but in different plant hosts (Moodley *et al*., 2014; Wang *et al*., 2013). PVY belongs to the family *potyviridae* which is the largest plant virus family. It has a monopartite positive single stranded (+) ssRNA genome and belongs to the genus potyvirus (Coutts and Jones, 2015). Phylogenetic analysis in the current study revealed a high homology of above 90% comparing the nucleotide sequence of the three strains. Similar studies indicate that using the amino acid sequences of the Coat protein (CP) of the virus species, the genus potyvirus exhibit 38-47% similarity while strains share 90-99% similarity (Moodley *et al*., 2014).

The African nightshade was highly infected with PVY^{NTN} (necrotic strain) causing veinal necrosis on infected plants. Similar symptoms have been reported in other solanaceous crops. The virus has been reported to be responsible for 50% yield losses in potato and as the most intercepted virus worldwide (Gutierrez *et al*., 2013; Wang *et al*., 2016). The PVY was prevalent in all the agro ecological zones (UM2, LH2, UM4 and LM2) sampled, which indicates the economic importance of the virus in Kenya and the need for its surveillance. This report indicates that the most productive regions where ANS is produced have high incidence of PVY.

Cucumber mosaic virus belongs to the genus *Cucumovirus* with 60-65% strains identity and is one of the most common plant viruses of major agricultural significance (Jacquemond, 2012). It is a tripartite virus having three plus sense, single stranded RNA molecules encased in separate particles (Jacquemond, 2012). RNA1 and RNA2 encodes for the protein 1a and 2a, respectively which forms the replicase complex (Thompson *et al*., 2015). The Kenyan isolates revealed up to 86 to 96% similarity to 10 isolates worldwide. Kenyan CMV isolates caused variable symptoms, including necrotic or chlorotic lesions, mild to severe mosaic, stunting, leaf deformation and shoestring formation. Similar symptoms were reported by Rabie *et al*. (2017). Phylogenetic analysis revealed isolate homology with 10 different isolates of CMV from Kenya, China, South Korea, South Africa and Brazil at the nucleotide level. At the amino acid level, 1a protein showed close resemblance to Kirinyaga isolates (MH567342.1 and MH567352.1) from Kenya. The amino acid determines whether the strain induces necrotic lesions or systemic mosaic on plants (Jacquemond, 2012). Based on RNAI sequence phylogenetic analysis, CMV isolate in this study was found to be closely related to that of S. Korea and that of China.

This study reports high prevalence of the virus in Kenya as also reported by Mutuku *et al*. (2018). Subgroup I as shown in the phylogenetic tree was the predominant strain and can be further subdivided into 1A and 1B Jacquemond, (2012) and Thompson *et al*., (2015). Subgroup I strains shows severity in terms of symptom and disease development in tobacco (Tungadi *et al*., 2017). Similar reports show CMV subgroup I to be competitive in its infection on different host plants (Tungadi *et al*., 2017). Due to close resemblance of KM047509.1 at 97% nucleotide, RNAI amino acid and worldwide distribution with the African nightshade in the current study, it can be deduced that the CMV isolate can be placed in subgroup I. Based on the phylogenetic studies, the African nightshade is predominantly infected with CMV subgroup I.

Tobacco mosaic virus (TMV) belongs to genus *Tobamovirus* and has a very wide host range, it can cause serious economic impact in many crop families such as cucurbits, brassicas, solanaceous and ornamental plants (Adams *et al*., 2009). The infected plants showed different type of symptoms which included mosaic, malformation, mottle and stunting. TMV infections have been reported in Kenya on solanaceous crops but no reports on African nightshade (Adams *et al.,* 2009). Phylogenetic analysis of TMV in African nightshade isolates revealed its wide distribution in agro ecological zones UM2, LH2, UM4 and LM2 with 50% of the samples analyzed using RT-PCR testing positive for TMV. The homology of nucleotide and amino acid sequences analysis ranged from 87-98% and 98.2-99.4%, respectively. *Tobacco mosaic virus* was closely related to isolates from European and Asian countries showing a worldwide distribution. Kreuze *et al*. (2009) and Wang *et al*. (2016) also reported worldwide distribution of TMV.

Tobacco mosaic virus identified in the current study is closely related to those isolated from tobacco, tomato and potato. TMV strains have genetic variation in different hosts and environmental conditions (Ho and Tzanetakis, 2014). The presence of TMV in greenhouse samples in the current study indicates that it is possible for the virus to perpetuate itself through germplasm. Similar studies have reported that *Tobamovirus* can easily be transmitted mechanically through seed and contact between plants, but not transmitted by a vector (Kreuze *et al*. 2009) and that debris can be an important source of inocula in the fields (Wang *et al*. 2011). This study deployed molecular techniques for detection and characterization of the viruses associated with African nightshade. That was in recognition of the difficulties of using symptoms to identify viruses that usually occur in multiple infections under field conditions.

CHAPTER SIX

EFFECT OF PROCESSING METHOD ON QUALITY OF AFRICAN NIGHTSHADE SEED

6.1 Abstract

African nightshade (*Solanum scabrum* and S. *villosum* Miller) seed production is hampered by the poor processing methods used by farmers. The objective of this study was to evaluate seed processing methods used by farmers and recommend the most suitable for production of quality African nightshade seed. To evaluate seed processing methods used by farmers, seeds that were collected during a field survey were subjected to quality tests. Seed quality tests were done at the University of Nairobi Kabete Campus and field evaluation experiments were set up in Muthara location Meru County under controlled environment. Four seed processing methods namely dry seed processing, wet seed processing, dry seed fermentation and wet seed fermentation processing were evaluated and were significantly ($p \leq 0.05$) different. Seed processing method was a major determinant of seed quality which in turn determines crop growth vigor and yields. Evaluation of seed processing methods revealed that, wet seed fermentation method produced seeds with the highest seed purity of 96.3% and highest yields (913.8kg/ha). Principal component analysis showed a significance ($p \leq 0.05$) positive correlation between seed purity, growth parameters and crop yields. Therefore, this study recommends wet seed fermentation method for use by farmers since it leads to seed of high quality with high germination and growth attributes.

6.2 Introduction

Cultivation and consumption of African nightshade has continued to increase due to the significant role it plays in nutrition, food security and income generation (Abukutsa-Onyango, 2010). The indigenous vegetable is traded at a higher price compared to exotic vegetables due

to increasing demand (Abukutsa-Onyango, 2010). Lack of quality seeds is a major constraint to production of the vegetable. Poor quality is responsible for low yields for example in Kenya, nightshade leaves have the potential to yield 30 tonnes but farmers attain less than 2 tonnes per ha (Elizabeth and Adeniji, 2015). Seed processing method is a major determinant of the seed quality. Lack of knowledge on processing of African nightshade seed has led to seed stocks of poor quality (Ekhuya *et al*., 2018). Solanaceous crops where fruits are borne on succulent berries are better processed by wet fermentation method (Milosevic, 2010). Wet seed fermentation ensures clean seed production that is free from physical impurities due to washing and removal of sugars that inhibit seed germination (Ekhuya *et al*., 2018).

Seed quality is determined following rules by ISTA (International Seed Testing Association) and AOSA (Association of official seed analysts). The germination test is the most widely accepted physiological method for testing the quality of seed (Milosevic, 2010). According to Barros *et al*. (2002) and Santorium (2013) the results of the germination test overestimates the physiological potential of the seed, as it is conducted under optimal conditions. Therefore, there is need for seed vigor tests to predict the performance of seeds in the field (Milosevic, 2010). In addition, growth tests of high quality seeds produce plants that are normal, vigorously growing with low sensitivity to external factors (Milosevic, 2010). Farmers' process own seeds in Kenya using different methods and thus lack a uniform, standard and optimal method for processing seeds.

6.3 Materials and methods

6.3.1 Experimental site

The seed samples used in this study were obtained from experimental sites as described in section 3.3.1.

6.3.2 Survey and seed collection

Survey design and seed collection procedures were as described in section 3.3.2 to 3.3.3. A total of 120 seed samples that were collected from farms were used in this study. The following methods were used by farmers to process farm saved seeds: wet seed fermentation, dry seed fermentation, wet seed processing and dry seed processing. The methods were not standard and farmers slightly modified them but the most critical aspects like fermentation and use of water were crucial in categorization. The following were the seed processing methods as described by farmers. Wet seed fermentation involved seed extraction and fermentation of seeds by placing them in water for 2 to 4 days. This was followed by seed cleaning and drying. Dry seed fermentation involved seed extraction and fermentation without use of water by placing them in woven sacks or polythene bags for periods ranging from 5 to 14 days. This was followed by seed drying and removal of chaff. Wet seed processing entails extracting the seeds in water to remove fruit pulp followed by seed cleaning and drying. Dry seed processing involves extracting the seeds and drying them together with the chaff. In addition, some farmers considered seed processing tedious and time- consuming and did not process their seeds. Instead they uprooted old nightshade plants with berries and placed them in the garden to germinate.

6.3.3 Seed quality tests

Seed quality tests were done in the laboratory on the seeds processed using different methods. The following tests were carried out: seed moisture content, germination percentage, seedling

emergence, seedling length and seedling vigor index as described in section 3.3.3 and 3.3.4. In addition, germination index and time to 50% germination was determined. Germination index (GI) was calculated as described by Association of Official Seed Analysts (AOSA, 1983) by the following formula.

$$GI=\frac{\text{No. of germinated seed}}{\text{Days of first count}}+\frac{\text{No. of germinated seed}}{\text{Days of final count}}$$

Time for 50% of germination (t ½/T_{50}), is the point of the distribution in which the mean, medium or mode (point at which the highest frequency of germinated seeds) is observed. It represents the peak of germination. The time to 50% germination (T50) was calculated according to the formula of Coolbear *et al*., (1984) modified by Farooq *et al*., (2005).

$T_{50} = ti + \{(N_{/2})\text{-}ni\}\ [ti\text{-}tj][ni\text{-}nj]$. Where N is the final number of germination and ni, nj are cumulative number of seeds germinated by adjacent counts at times ti and tj when $ni<N/2<nj$.

6.3.4 Field experiment

The field experiment was conducted during short rains (SR) of October-November, 2018 in Muthara location Meru County. Field sowing was done on 18th October, 2018 using farm saved seeds obtained from four agro ecological zones (UM2, LH2, UM4 and LM2). Seeds were processed following different methods including wet seed processing, dry seed processing, dry fermentation and wet fermentation were sown in different plots. A randomized complete block design (RCBD) with four treatments (seed processing methods) and four replications, separately for each experimental sites were adopted. The treatments were arranged in split-plot design. Agro-ecological zones were the main plots (UM2, LH2, UM4 and LM2) and the processing methods represented the subplots. The plot size was 3x3m^2 with spacing of 60cm by 30cm and two seeds per hill. Two manual cultivations were done to control weeds and

supplementary irrigation was done when needed. No pesticides were applied and all standard agronomic practices were carried out for all plots for uniformity.

6.3.5 Field data collection

Data was collected on stand count, days to 1st flowering, plant height, number of fruits per plant and seed yields. The stand count was recorded by counting the number of plants that emerged in each plot and the data converted to plants/hectare. Plant height was determined by measuring the height of 10 randomly selected plants per plot. Ten plants from each plot were tagged and then fruits of each harvest were cut to evaluate seed processing methods. For seed yield data, the fruits were harvested separately in each plot, processed and weighed with an electronic balance to determine seed yield. The seed weight was evaluated with values corrected for 10% moisture content. All seeds were air dried to a constant moisture content of 10% and then kept in temporary storage at 20^0C during initial testing. A determination of moisture content (fresh weight basis) was done using seed moisture meter (GMK-310 RT, G-Won Hitech Co. Ltd).

6.3.6 Evaluation of processing methods under controlled environment

Seeds were planted in the field to evaluate the seed processing methods. The experimental design and agronomic practices followed the description given in section 6.3.4. Ten plants were tagged in each plot using different color tags for each processing method. On reaching maturity the plants were harvested; fruits of each harvest were cut and processed following standard methods. The following procedures were adapted and modified and adopted using indigenous knowledge obtained from farmers during the survey on seed processing and the standard procedures as described by (Colley *et al.*, 2015 and ISTA, 2014).

6.3.6.1 Wet seed processing

This method involved removing the seed from the overripe fruit, crushing them by hand in a basin or inside a bag. A jar of water was used to separate seeds from debris. Seeds usually sink and debris floats. This was followed by removing debris through repeated washing, and then drying.

6.3.6.2 Dry seed processing

The method was carried out by allowing the seeds to ripen on the plant, after which the fruits were harvested and then dried. The fruits were crushed together with the pulp and seeds were manually separated from the fruits chaff through sieving and winnowing.

6.3.6.3 Dry seed fermentation

This method carried out by harvesting of ripe nightshade fruits, they were then crushed and the mixture was placed in woven sacks without adding any water for a period of 5 days. The mixture was then dried. The mixture was manually separated to remove the chaff from seeds through winnowing.

6.3.6.4 Wet seed fermentation processing

Fully ripe fruits were opened by hand or gently squeezed while inside a woven bag. A mixture of seeds, pulp and juice obtained was cleaned by hand washing. The dirt and debris was rinsed from the mixture. The remaining mash of seeds, pulp and juice were poured in a basin or bucket. The mixture in a basin or bucket was fermented up to 3 days at an ambient temperature of 25^0C. The mixture was stirred three times a day for aeration and even fermentation. During fermentation, the scum that appeared on the top of the mixture indicated that the process was successfully taking place. The top layer of the scum and pulp was poured off after 3 days of fermentation. Water was poured into the remaining mixture so that the volume was doubled.

The mixture was stirred and allowed to settle again, and the top layer of pulp and debris poured off. Some lighter, less viable, seed were poured off with top layer. The process was repeated severally until the water was fairly clean. The remaining contents (seed) were poured through a strainer retaining the seed and draining off the remaining water. The seeds were spread on woven or sack mats to dry. They were turned severally for even drying under a shade. Processed seed samples were put in brown paper (khaki) bags and kept at 5^0C cool storage cabinet at the University of Nairobi awaiting seed quality and germination analysis. Seed quality tests were done as described in section 3.3.3 and 3.3.4.

6.3.7 Data analysis

Data was presented using tables and graphs. The mean values for seed quality and germination tests, were used for statistical analysis using statistical software version 9.2 (SAS; 2002). Principal component analysis (PCA) and Pearson's correlation coefficient were used to infer relationships to determine the most suitable seed processing method. Means separation was done using least significant difference (LSD) test at ($p \leq 0.05$) probability level.

6.4 Results

6.4.1 Seed processing methods in agroecological zones

In order to obtain African nightshade seeds, farmers process berries using different methods that were recorded. These methods generally follow a similar process of seed extraction, cleaning and drying. The processing methods used include dry seed processing, wet seed processing, dry seed fermentation and wet seed fermentation. The farmers in each agro-ecological zone preferred a particular method of processing and there were significant ($p \leq 0.05$) differences in the method of processing in the agro ecological zones. In UM2, 36% of farmers preferred dry seed fermentation being the highest and the least frequently used method being

wet seed processing at 12%. In LH2, the method with the highest frequency was dry seed fermentation (38%) while the lowest was wet seed processing at 10%. However, in UM4 and LM2, wet seed processing was the most preferred with 47% and 42%, respectively and the least preferred in both zones was dry seed fermentation at 17% each (Figure 6.1). The choice of seed processing method was not dependent on the gender of the farmer and both gender had a preference for wet seed processing method with 44% for males and 43% for females. Similarly, wet seed fermentation was the least preferred by males at 9% and females 12% (Figure 6.2).

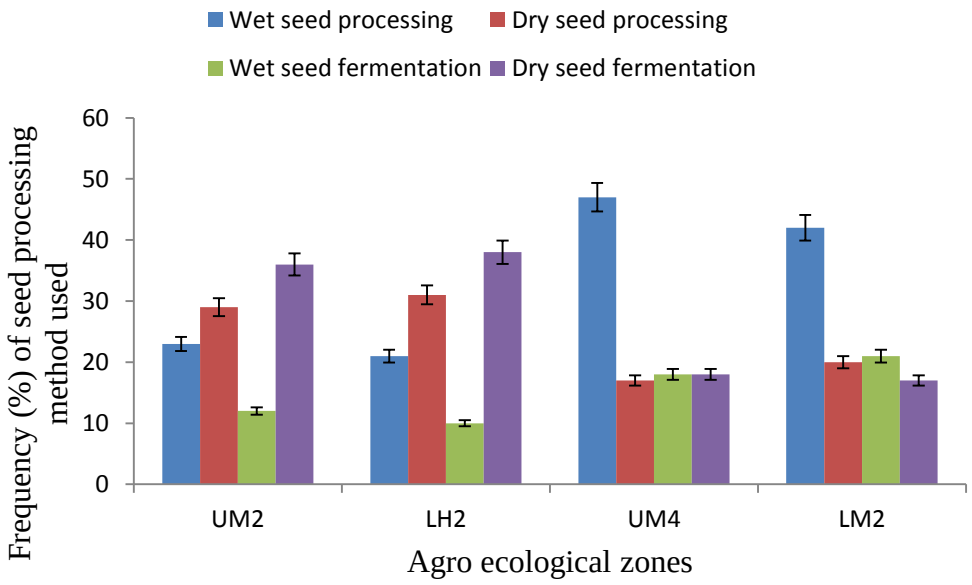


Figure 6.1 Percentage frequency of seed processing method preference in each agro ecological zone

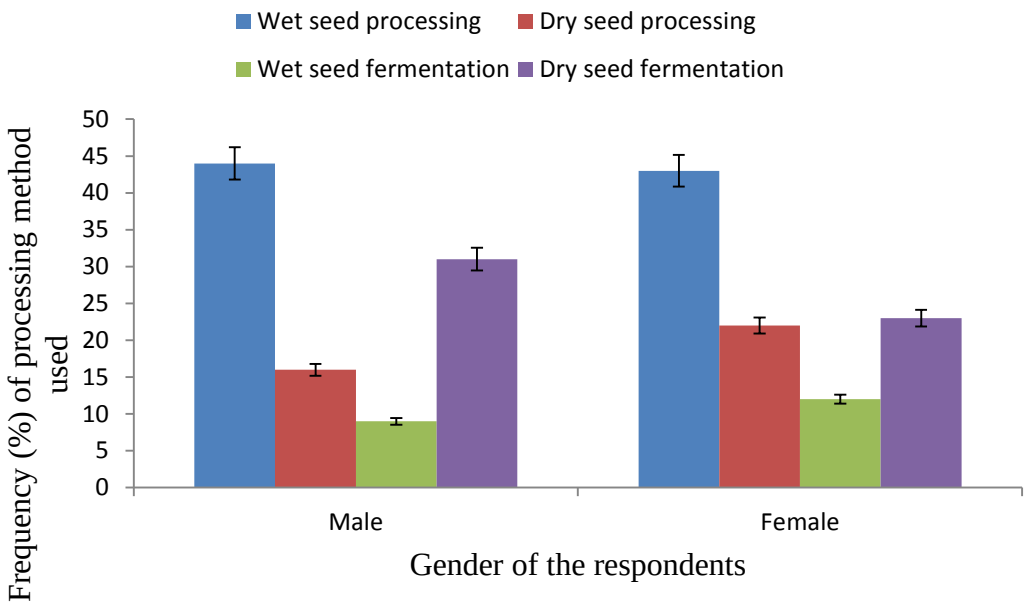


Figure 6.2 Percentage frequency of seed processing method preference by gender of respondents

Wet seed processing was the most prefered method across all the ages while the least preferred was wet seed fermentation (Figure 6.3). In addition, the method of seed processing used was not dependent on the level of education. The most prefered method by all farmers at different levels of education was wet seed processing at 36% and the least preferred was wet seed fermentation 15% (Figure 6.4)

6.4.2 Growth tests for farm saved seeds

Moisture content (MC) level was significantly ($p \leq 0.05$) higher in farm saved seed in different processing methods. The MC was higher in wet processed seed at 11.1% and lower in dry processed seed (8.6%). Germination percentage, germination index and seedling emergence were higher in wet seed fermentation at 89.2, 18.2 and 91.2% respectively, compared to the dry seed processing method which recorded 80.3, 11.2 and 82.4%, respectively (Table 6.1). Comparing time to 50% germination, seeds under wet fermentation took less than 3 days while seeds under dry seed fermentation took more than 4 days. Seedling vigor index, a key

germination parameter, was highest in wet seed fermentation at 347.9 and the lowest was recorded for seeds processed by drying at 303.1 (Table 6.1).

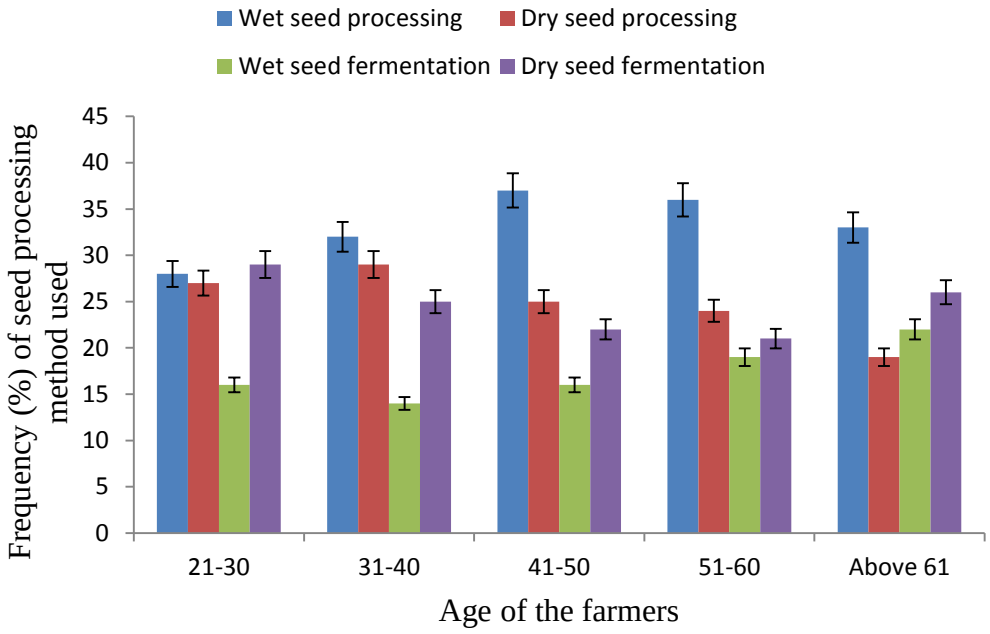


Figure 6.3 Percentage frequency of seed processing method preference by age of the farmers

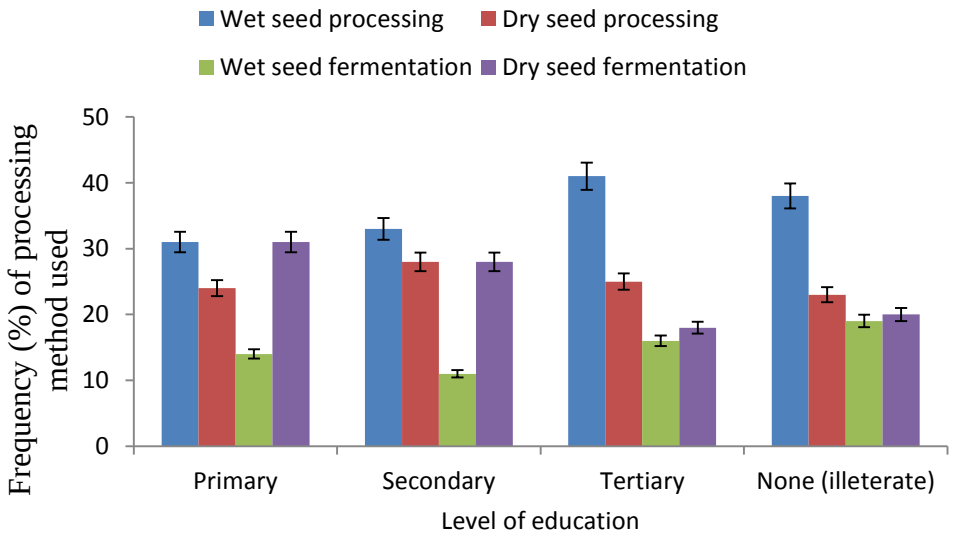


Figure 6.4 Percentage frequency of seed processing method preference by level of education

Table 6.1: Moisture content, germination and growth tests for seeds processed using different seed processing methods under laboratory conditions.

Seed processing method	MC	GP	GI	T_{50}	SE	SL(cm)	SVI
Wet seed processing	11.2^{c}	81.4^{a}	13.2^{ab}	3.3^{a}	83.6^{a}	3.6^{a}	293.0^{a}
Dry seed processing	8.6^{a}	80.3^{a}	11.2^{a}	4.1^{c}	82.4^{a}	3.8^{a}	305.1^{ab}
Wet seed fermentation	9.2^{a}	89.2^{c}	18.3^{c}	3.2^{a}	91.2^{c}	3.9^{b}	347.9^{b}
Dry seed fermentation	10.2^{b}	84.2^{b}	14.3^{b}	3.7^{b}	86.7^{b}	3.6^{a}	303.1^{a}
[1]Lsd ($p \leq 0.05$)	0.9	2.3	2.6	0.24	1.8	0.2	44.3
[2]CV (%)	13.4	24.5	19.3	19.4	31.6	8.8	12.6

[1]Least significant difference and [2]Coefficient of variation. Means followed by the same letter (s) within columns are not significantly different ($p \leq 0.05$). Means are separated by LSD ($p \leq 0.05$). MC-Moisture content, GP- Germination percentage, GI- germination index, T50-time to 50% germination, SE-seedling emergence, SL-seedling length and SVI- Seedling vigor index.

Field data on various parameters had significant differences. Stand count was highest in wet fermented processed seeds at 94% while for dry processed seeds was at 85% (Figure 6.5a). The plant height was highest in wet processed seeds (78.2 cm) and lowest in dry processed seed (74.2cm) (Figure 6.5b). Seed processing methods had no significant effect on the time of the first flowering with plants flowering almost at the same time (Figure 6.6a). Seed processing did not have a significant effect on the number of branches bearing fruits per plant (Figure 6.6b). However, the processing methods had significant ($P<0.05$) variability in the number of fruits per plant and seed yields (kg/ha). Wet seed fermentation had the highest fruits per plant and seed yields/ha recording 98% and 913.8 kg/ha, respectively and the lowest was recorded in wet seed processing with 90% and 622.5kg/ha (Figures 6.7 a & 6.7b).

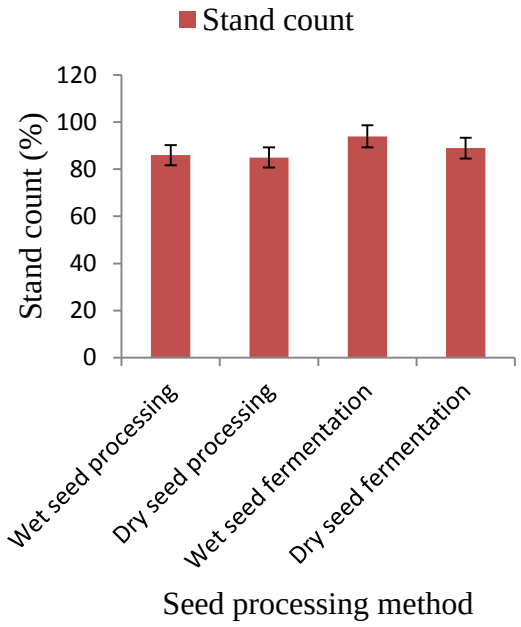


Figure 6.5a: Stand count for seed processed by different methods

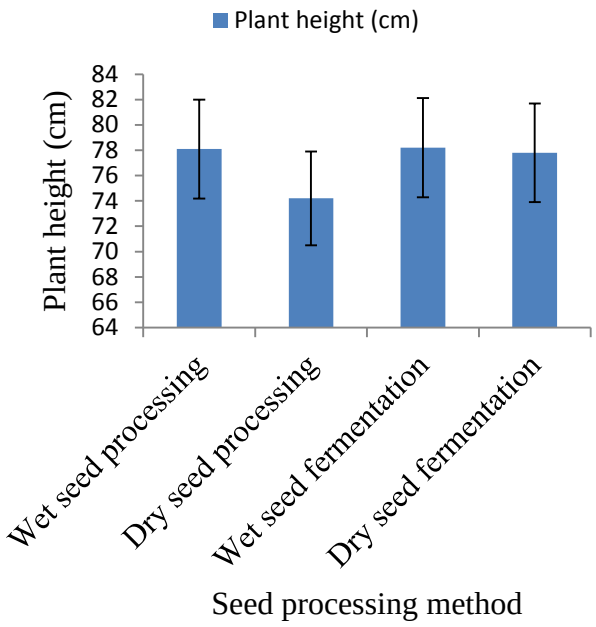


Figure 6.5b: Plant height (cm) for seed processed by different methods

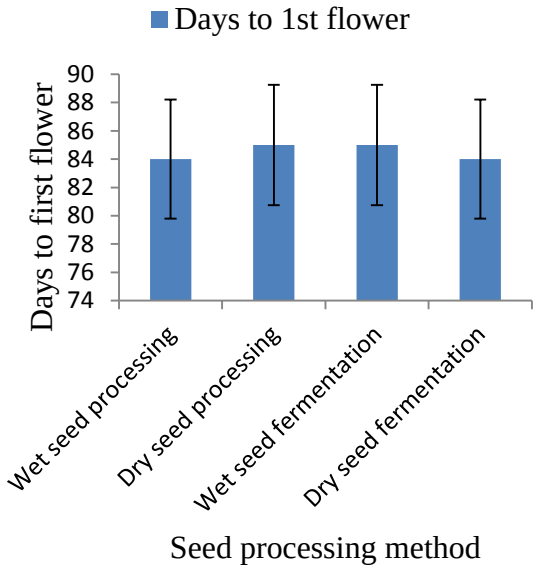


Figure 6.6 a: Days to first flower for seed processed by different methods

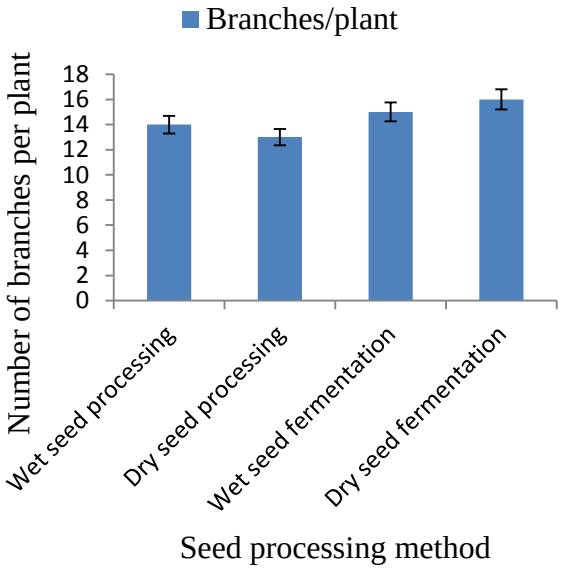
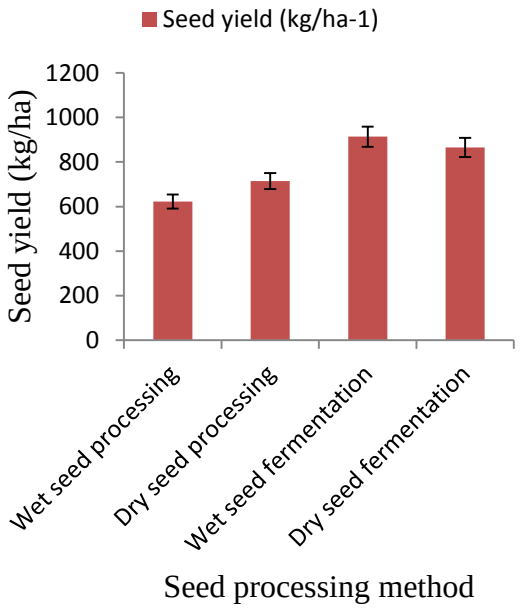


Figure 6.6b: Branches per plant for seed processed by different methods



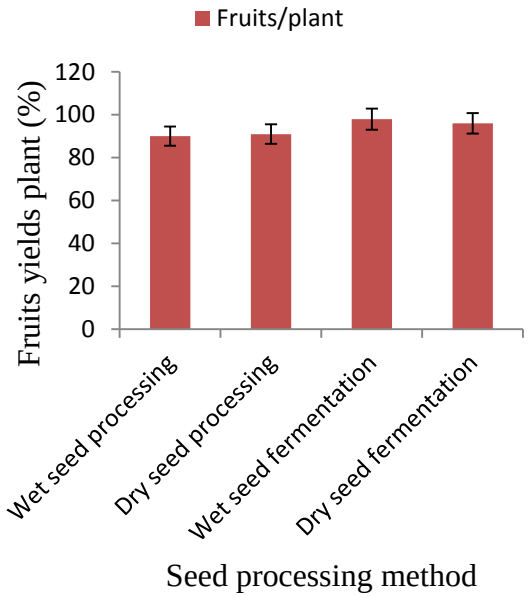


Fig. 6.7a: Seed yields (kgs/ha) for seed by different methods

Fig. 6.7b: Fruits per plant for seed processed by different methods

6.4.3 Seed purity and growth tests obtained for field experiment

On subjecting seeds harvested and processed to purity tests, wet fermentation processing method had the highest purity of 96.3% and low in content of other crop seeds, inert matter, discolored seeds and no insect damage. Dry seed fermentation produced seeds with the lowest purity of 72% and was high in content of other crop seeds, inert matter, discolored seeds and insect damage (Table 6.2). Comparing growth tests for the different processing methods, there were significant differences on the various parameters tested. Wet fermentation processed seeds exhibited a high level of seed quality; germination percentage (GP) was 97.2%, seedling vigor index (SVI) 408.2 and other parameters followed a similar trend (Table 4.2). Dry seed processing method had the lowest quality with the lowest in all growth parameters. Germination percentage (GP) and seedling vigor index (SVI) recorded 84.1% and 319.6, respectively (Table 6.3).

Table 6.2: Quality parameters of African nightshade seed processed using different methods in percentage.

Seed processing method	Pure seed	Other crop seeds	Inert matter	Discolored seeds	Shrivele d seed	Insect damage d
Wet seed processing	87.5[a]	2.8[b]	5.0[b]	2.7[b]	1.4[b]	0.6[c]
Dry seed processing	76.4[b]	4.3[a]	6.3[b]	6.9[a]	4[a]	2.1[b]
Wet seed fermentation	96.3[a]	0.5[c]	1.2[c]	1.2[b]	0.8[b]	0.0[c]
Dry seed fermentation	72.0[b]	4.2[a]	10.3[a]	5.5[a]	4.2[a]	3.8[a]
[1]Lsd ($p \leq 0.05$)	10.6	1.2	2.8	2.4	1.3	1.2
[2]Cv%	24.3	19.3	14.2	22.1	17.4	32.1

[1]least significant differences and [2]coefficient of variation. Values are the means, each having three replicates. Means followed by the different letter(s) within columns are significantly different; means are separated by LSD ($p \leq 0.05$).

Table 6.3: Seed moisture content, germination and growth tests for seeds processed using different methods under laboratory conditions.

Seed processing	**MC**	**GP**	**GI**	**T50**	**SE**	**SL (cm)**	**SVI**
Wet seed processing	10.2[a]	84.2[b]	14.2[b]	3.6[b]	84.4[b]	3.9[b]	328.4[b]
Dry seed processing	8.3[b]	84.1[b]	13.8[b]	4.1[a]	84.1[b]	3.8[b]	319.6[b]
Wet seed fermentation	9.6[a]	97.2[a]	20.1[a]	3.4[b]	93.1[a]	4.2[a]	408.2[a]
Dry seed fermentation	8.1[b]	92.1[a]	18.2[a]	4.2[a]	88.8[a]	3.7[b]	340.8[b]
[1]Lsd ($p \leq 0.05$)	1.2	5.4	3.8	0.24	6.8	0.2	32.3
[2]CV (%)	23.1	31.2	19.2	27.2	31.6	8.8	31.7

[1]Least significant difference. [2]Coefficient of variation. Means followed by the different letter (s) within columns are significantly different at LSD ($p \leq 0.05$). MC: Moisture content, GP: Germination percentage, GI: Germination Index, T50: Time to 50% germination, SL: Seedling length and SVI: Seedling Vigor Index.

Principal component analysis (PCA) was carried out with four germination parameters to determine their effect on seed processing method. Seed purity was the most important parameter as it showed excellent correlation with seedling vigor index, germination percentage and yields per ha (Figure 6.8). Seed processing method contributed to maximum variance of 93.59% and growth factors contributing to a variance of 6.22% on Y-axis (Figure 6). The first principal components were seed purity and seedling vigor index and these strongly correlated with germination percentage and yields per hectare (Figure 6.8).

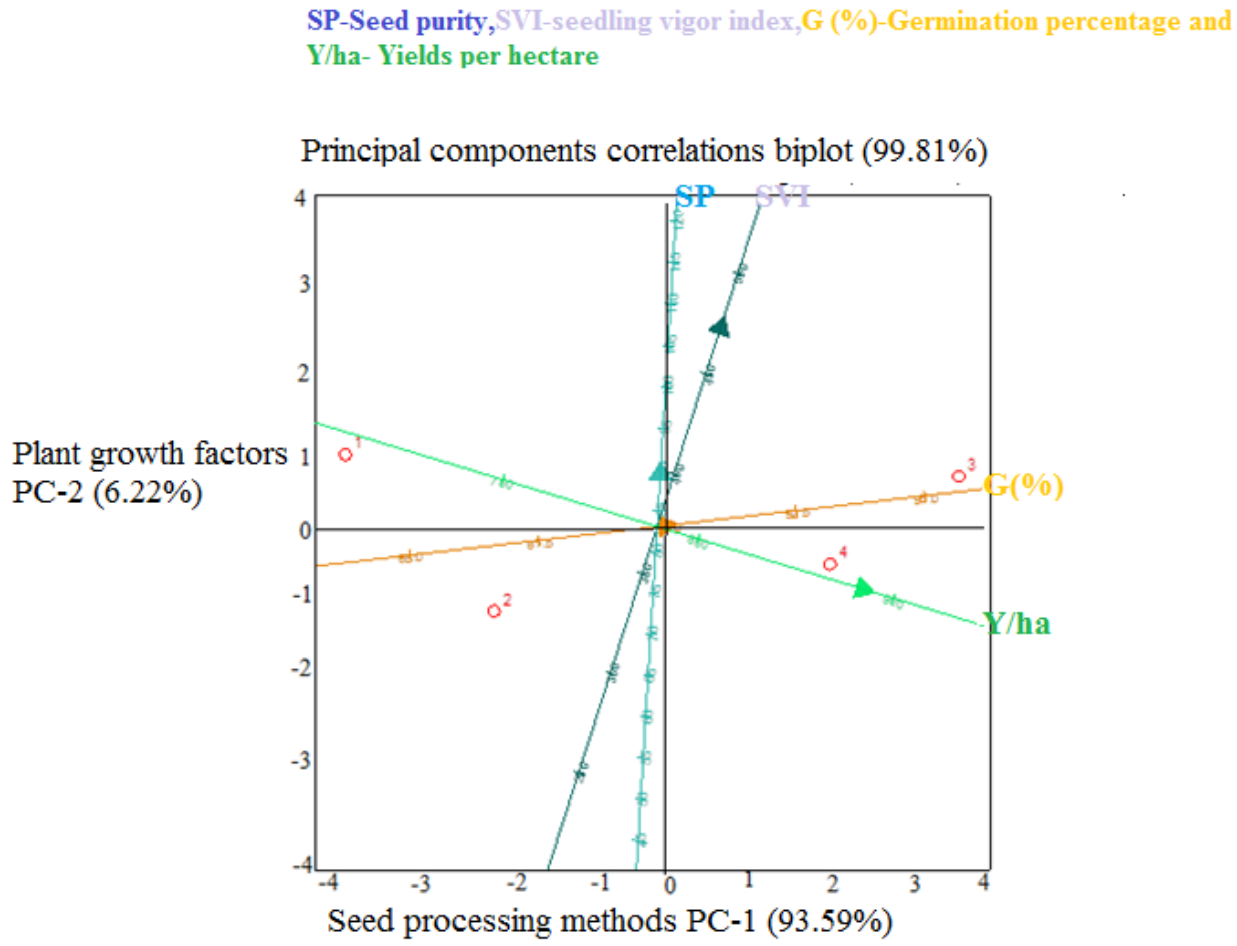


Figure 6.8: Principal component analysis (PCA) of African nightshade seed germination parameters under different treatment methods.

There was a positive and significant correlation between seed and crop growth parameters (Table 6.4). Seed purity had a positive correlation with germination percentage (r=0.922), germination index (r=0.855), seedling vigor index (r=0.811) and yields per hectare (r=0.828). Germination percentage was positively correlated with seedling vigor index (r=0.756) and yields per hectare (r=0.766). Germination index was positively corrected to germination percentage (r=0.867) and seedling vigor index (r=0.723). Seedling vigor index was positively correlated with seed purity (r=0.9130), germination percentage (r=0.898) and germination index (r=0.766). In addition, crop yield was positively influenced by seed purity (r=0.922), germination percentage (r=0.913) and seedling vigor index (r=0.782) (Table 6.4).

Table 6.4: Correlation among different germination parameters and seed processing methods.

Characters	SP (%)	GP (%)	GI	SVI	F/P	Y/HA
SP (%)	1	0.922**	0.855**	0.811*	0.125	0.828**
GP (%)	0.666**	1	0.635	0.756*	0.526	0.766*
GI	0.433	0.867**	1	0.723*	0.245	0.568
SVI	0.913**	0.898**	0.766*	1	0.332	0.968**
F/P	0.322	0.831*	0.327	0.327	1	0.748
Y/HA	0.922**	0.913**	0.388	0.782*	0.234	1

SP: Seed Purity, GP: Germination percentage, GI: Germination Index, SVI: Seedling Vigor Index, F/Fruits/Plant and Y/HA: Yields per hectare. *, ** Significant correlation at $p \leq 0.05$ and $p \leq 0.01$ respectively.

6.5 Discussion

This study revealed that four methods of seed processing are variably preferred by farmers in different agro-ecological zones to process edible nightshade seed. These methods were wet seed processing, dry seed processing, dry seed fermentation and wet seed fermentation. In addition, some farmers preferred to have plants mature and ripen in the field, uproot and place them in the garden to dry and germinate without any processing. Variation in processing method preference in the agro ecological zones was attributable to the indigenous knowledge passed on from one generation of farmers to the next. Due to poor fermentation farmers reported rotting of berries which has also been reported by Ekhuya *et al.,* (2018). It is recommended that fermentation should be done under controlled environment and seeds washed severally before drying (Amaza *et al.,* 2010), in order to remove germination inhibitors (Oiye *et al.,* 2009). However, in UM4 and LM2 agro ecological zones majority of the farmers preferred wet seed processing as the method is fast and less tedious without need to wait for seeds to ferment. This shows that farmers are not aware of the benefits of proper seed fermentation in quality seed production. Farmers planting low quality seed risk poor field emergence and low plant vigor as a result of poor physiological quality of seed (Mathews *et al.,* 2012).

Seed washing is a critical step in seed processing and was done by more than half of the farmers' sampled. Washing seeds with water facilitates removal of sugars in the nightshade fruit pulp that causes seed dormancy leading to low vigor and poor yields. Elizabeth and Adeniji (2015) reported low vigor of indigenous vegetable seeds caused by inadequate removal of sugars and germination inhibitors. The method of processing was mainly determined by the indigenous knowledge passed on from previous generation. However, it was not influenced by gender, age or the level of education. Elizabeth and Adeniji (2015) reported that many farmers experienced difficulties in seed processing and lacked a standard processing procedure leading to poor quality seed. Abukutsa (2010) reported that farmers lack knowledge on processing of indigenous vegetable seeds because of lack of training or weak extension services.

Wet seed fermentation processing resulted in seeds with high germination and field parameters resulting in high yields per hectare. This method recorded high germination percentage, seedling vigor index and high seed yields per hectare. This could be attributed to high seed quality brought about by the removal of sugars that cause dormancy, pulp and other materials which inhibit germination. The high quality of seed resulting from this method was due to frequent washing to remove the pulp and juice and after fermentation, removal of the scum. The method also allows removal of plant debris and other impurities and proper separation of seeds of different densities. Dry fermentation does not involve washing of seeds leading to high level of impurities in seeds. Dry and wet seed processing are done without fermentation leading to poor quality of seeds. Oiye *et al.* (2009) reported that fermentation helps to break germination inhibition by removing the mucilage in the seeds.

Proper drying, packaging and storage of seeds are critical in maintaining seed quality. Ekhuya *et al*. (2018) reported that rapid sun drying affects the quality of seeds. Packaging of seeds is

important to prevent damp conditions leading to poor quality seeds. Farmers reported that seeds can remain viable for many months when kept dry, but rapidly lose germination capacity when stored in humid conditions. However, majority of farmers stored their seeds for shorter periods high demand for limited seeds, lack of good processing and storage facilities.

The principal component analysis revealed that seed purity is a key determinant of seedling vigor, germination percentage and yields per hectare. Seed purity had a strong positive correlation with germination parameters. Seed processing is the greatest contributor to seed quality. Low quality and vigor in crops has been reported on poorly processed seeds (Ekhuya *et al*. 2018). Farmers rely on these poorly processed seed from farms and market, leading to low germination and reduced yields. Accoring to Elizabeth and Adeniji (2015) more than 90% of farmers use own saved seeds which agrees with the findings in this study. This is mainly because limited efforts have been made by the government and seed companies to educate farmers or offer incentives to produce clean seeds of African nightshades.

CHAPTER SEVEN

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

7.1 General discussion

African nightshade (ANS) is an indigenous vegetable crop species that is widely cultivated and consumed in Sub-Saharan African countries. ANS leaves are consumed as food and its' popularity is increasing in rural, peri-urban and urban areas. The current prices of ANS are higher compared to exotic vegetables like kale and cabbage (Abukutsa-Onyango, 2010). African nightshade has become a common item in supermarkets and green grocery stores. Consumers prefer them because of high micronutrient content, medicinal properties and economic value (Abuktusa-Onyango, 2010; FAO, 2010). Farmers prefer to grow them as they require little financial input and the average gross margins are estimated to be US$ 3,033 per hectare compared to US$ 1,760 for kale (Mumbi *et al*. 2006).

Production of ANS is limited by poor quality seeds or scarcity of certified seeds. The seed system is not organized and largely informal with farmers producing their own seeds. This study has confirmed that farm saved seed is of poor quality and is likely to be highly infected with pathogens. Seed producers rarely test the seed for quality and pathogen infection. Farmers lack knowledge on how to prepare and process the seed relying on the indigenous knowledge relayed by older farmers (ancestors) (Ekhuya *et al*., 2018). The farmer saved seed is of poor quality with high levels of impurities compared to certified seed. Despite the challenges, farm saved seed is still the most popular method of seed acquisition and is used by over 90% of the farmers surveyed. Farmers continuously plant farm saved seed which is of poor quality leading to lower germination percentage, stand count, seedling vigor index and yields. Farmers' main focus is to produce ANS as a vegetable and seed production is a secondary activity undertaken by fewer farmers and in smaller scale. This leads to increased demand for ANS seeds during

planting season due to inadequate seed stocks. Use of certified seed is constrained by cost, long distance to markets, lack of knowledge and inadequate supplies (Ekhuya *et al*. 2018).

This study confirmed that farm saved seed is infected with fungal, bacterial and viral pathogens and is in agreement with similar studies by Handiseni *et al.* (2008), Ismael (2010), Uma and Weseley (2013), Abbas *et al*. (2014) and Groves *et al*. (2016) on other solanaceous crops. This study further identified the three major viruses which were *Potato virus Y*, *Cucumber mosaic virus*, *Tobacco mosaic virus* using metagenomics.

This study identifies ISTA methods as being effective in seed quality testing. Fungal and bacterial incubation methods including blotter and agar plating were effective for routine detection of fungal and bacterial pathogens in seeds. Biochemical tests were done to confirm the identity of the bacterial pathogens. In addition, microscopy was useful for taxonomical identification of fungal and bacteria pathogens. Results are in agreement with reports by Ismael (2010), Kaur (2010), Uma and Weseley (2013) and Hamin *et al*. (2014).

The study revealed that serology and RT-PCR are effective methods for large scale screening of viral pathogens in field crops and can be utilized by seed producing companies. Proper sampling is needed to have a representative sample that will aid in determining whether the entire seed lot is infected or not. Hence the need for standards to certify ANS seed. These methods are relatively simple to use, reliable and useful when testing large samples (Ward *et al*. 2014). Screening in seed stocks is important to prevent inoculum build-up. This is done on ungerminated seeds to determine the incidence of virus transmission through seed to seedlings (Albrechtsen, 2006). The primers designed for RT-PCR in this study proved to be highly efficient, specific and sensitive in identification of viruses infecting nightshades. RT-PCR is

currently the most common method for detection of seed borne pathogens and thus supersedes conventional detection methods (Paylan and Gumus, 2013).

Metagenomics studies using next-generation sequencing (NGS) methods have greatly increased knowledge on viruses infecting a wide range of hosts. With this method, it is possible to identify and characterize pathogens at the molecular level without previous sequence knowledge and because of the high volume of data generated (Ho and Tzanetakis, 2014). In the present study, *Potato virus Y* (PVY), *Cucumber mosaic virus* (CMV) and *Tobacco mosaic virus* (TMV) were sequenced for metagenomics study. Consensus sequences were aligned, gaps and missing data removed and the resultant consensus sequence deposited in the Genebank. The species revealed a high homology with a number of isolates worldwide. The usefulness of Next Generation Sequencing (NGS) as a virus diagnostic tool in certification programmes is limited by its high cost though it is sensitive and useful in detecting novel variants or virus species (Coutts and Jones, 2015).

Evaluation of seed processing methods identified wet seed fermentation as the most appropriate method that yielded seeds of high quality and yields. The high quality of seeds was due to fermentation and frequent washing to remove germination inhibitors and impurities.

7.2 Conclusion

The African nightshade farm saved seed and that sold in the markets by farmers was of poor quality and infected by fungal, bacterial and viral pathogens. The major fungi infecting the seeds were *Alternaria solani, Fusarium solani, Fusarium oxysporum, Aspergillus niger, Aspergillus flavus, Penicillium chrysogenum* and *Curvularia intermedia*. The bacterial pathogens were *Xanthomonas campestris* pv. *vesicatoria* and *Pseudomonas syringae* pv.

tomato while the main viruses found infecting seed and plants in the field were *Potato virus Y*, *Cucumber mosaic virus*, and *Tobacco mosaic virus*. The pathogens were widely distributed in the agro ecological zones sampled.

The most prevalent viruses were *Potato virus Y* (PVY), *Cucumber mosaic virus* (CMV) and *Tobacco mosaic virus* (TMV). *Potato virus Y* was the most frequently isolated species with three serotypes PVY^{NTN}, PVY^{O} and $PVY^{N:O}$. The PVY^{NTN} necrotic serotype was the predominant type. *Cucumber mosaic virus* was the second most frequently isolated virus and phylogenetic analysis revealed that it belonged to CMV subgroup I isolates. This subgroup causes necrotic and severe mosaic in host plants. *Tobacco mosaic virus* (TMV) had the lowest frequency of isolation and the symptoms were clearly manifested including mosaic, malformation and mottling. Phylogenetic analysis revealed close resemblance of the virus to isolates from solanaceous crops. Four major seed processing methods are preferred by farmers; dry seed processing, wet seed processing, dry seed fermentation and wet seed fermentation. Wet seed fermentation processing method is the most efficient for clean quality seed production. Seed quality correlated strongly with crop vigor leading to increased yields.

7.3 Recommendations

From the findings the following recommendations were derived:

i. Farmers should be trained to produce clean seeds and use certified seed to increase African nightshade production. Seed quality testing and screening for pathogens in African nightshade should be a pre-requisite before seed certification.

ii. Farmers should be trained to use wet seed fermentation method in situations where certified seed is not readily accessible due to cost or other reasons.

iii. Plant breeders should embark on breeding African nightshade for resistance against diseases and vectors that are associated with African nightshade. In addition, there is need to improve the preferred species of African nightshade to increase seed production.

iv. Further research is needed to map the prevalence of diseases infecting African nightshade in Kenya and develop ways of mitigating them.

REFERENCES

Abang, A.F., Kouamé, C.M., Abang, M., Hanna, R. and Fotso A. K. 2014. Assessing vegetable farmer knowledge of diseases and insect pests of vegetable and management practices under tropical conditions. *International Journal of Vegetable Science* 20(3): 240-253.

Abbas, M.F., Hameed, S. and Rauf, C.A. 2014. Presence of new strain of *Potato virus Y* in Pakistan. *International Journal of Agriculture and Biology* 5:941-945.

Abukutsa-Onyango, M.O. 2007. The diversity of cultivated African leafy vegetables in three communities of Western Kenya. *African Journal of Food Agriculture Nutrition and Development* 7(3):1-8.

Abukutsa-Onyango, M.O. 2010. Strategic repositioning of African indigenous vegetables in the Horticulture Sector Résumé, 1413–1419pp.

Abukutsa-Onyango, M.O.2003. Unexploited potential of African indigenous vegetables in Western Kenya. *Maseno Journal of Education, Arts and Science*: 4:103-122

Adams, M.J., Antoniw, J.F. and Kreuze, J.2005.Virgaviridae: a new family of rod-shaped plant viruses. *Archives of Virology* 154:1967-72.

Ahmed, R.Z., Ibrahim, I.A.M., Hassan, H.M.S. and EL-Wakil, D.A.2013. Incidence of *Potato virus* Y strains and effect of infection on the productivity of potato tubers. *Journal of Agriculture and Veterinary Sci*ence 2:58-64

AICAD, 2003. Examination of the needs through the study of poverty reduction strategy papers for Kenya, Uganda and Tanzania. African Institute for Capacity Development and Japan International Cooperation Agency 5pp.

Albrechtsen, S.E.2006. Testing methods for seed transmitted viruses: Principles and protocols. CABL Publishing Oxfordshire, UK 259 pp.

Ali, A. and Kobayashim. L.2010. Seed transmission of *Cucumber mosaic virus* in pepper. *Journal of Virology Methods* 163 (2):234-237.

Ali, J.2012. Factors influencing adoption of postharvest practices in vegetables. *International Journal of Vegetable Science* 18 (1):29-40.

Alverez, J.M. and Hutchinson, P.J.S. 2005. Managing hairy nightshade to reduce potato viruses and insect vectors. *Pest Management Journal* 16:249-252.

Amaza, P., Udoh, E., Abdoulaye, T. and Kamara, A. 2010. Analysis of technical efficiency among community-based seed producers in the savannas of Borno State, Nigeria. *Journal of Food, Agriculture and Environment* 8:73-79.

Ambrose-Oji, B.2012. Urban food systems and African indigenous vegetables: defining the spaces and places for African indigenous vegetables in urban and peri-urban agriculture. *African indigenous vegetables in urban agriculture* 1:34.

Babiker, A. Z., Dulloo, M. E., Mustafa, M. A., Balla, E. and Ibrahim, E. T. 2010. Effects of low cost drying methods on seed quality of *Sorghum bicolor* (L.) Monech. *African Journal of Plant Science* 4(9):339-345.

Babu,B.,Hedge,V.,Makeshkumar,T.and Jeera,M.L.2012.Rapid and sensitive detection of potyvirus infecting tropical tuber crops using genus specific primers and probes.*African Journal of Biotechnol* 10:1023-1027.

Baldauf, P.M., Gray, S.M and Perry, W. 2006. Biological and serological properties of *Potato virus Y* isolates in North Eastern United states *Journal of Plant disease* 90:5.

Barros, D., 2002. Comparing quality tests for vigor parameters in tomato. *Brazilian Journal of Agricultural Sciences* 24:12-16.

Bennett, J. W. and Klich, M. 2003. Mycotoxins. *Clinical Microbiological Review* 16:497–516.

Carusso, P., Gorris, M.T., Cambra, M., Palomo, J.L., Collar, J. and Lopez, M.M.2002. Enrichment, double- antibody sandwich indirect enzyme-linked immunosorbent assay that uses a specific monoclonal antibody for sensitive detection of *Ralstonia solanacearum* in symptomatic potato tubers. *Applied Environment Microbiol*ogy 68:3634-3638.

Cervantes, F. A. and Alvarez, J.M. 2008. Role of hairy nightshade *Solanum sarrachoides* (sendtner) in the transmission of potato virus Y (PVY).strains by aphids and study of different PVY strains reaction in *Solanum tuberosum* (Linnaeus). *Journal of Phytopathology* 3:98.

Cetintas, R. and Yarba, M.M.2010. Nematicidal effects of five plant essential oils on the southern root knot nematodes (*Meloidogyne incognita* race 2). *Journal of Animal and Veterinary Advances* 9:222-310.

Chen, Y., Conner, R. and Gillard, C.L.2007. A specific and sensitive method for the detection of *Colletotrichum lindemuthianum* in dry bean tissue. *Plant Disease* 91:1271-1276.

Chowdhury, N., Ghosh, A.and Chandra G. 2008. Mosquito larvicidal activities of *Solanum villosum* berry extract against the dengue vector *Stegomyia aegypti*. *Journal of Alternative Medicine* 10:72-82.

Chowdhury, Z., Monjil, M.S. and Hossain, M.M.2005. Seed borne fungi in cucumber (*Cucumis sativa L.)* and melon (Cucumis melol.) of mynensingh, Bangladesh. *Journal of Seed Science and Technology* 9:105-109

Colley, M., Alex, S. and Linda, B. 2015. Organic seed processing: Threshing, cleaning and storage: Organic Agriculture handbook 354. Ohio state University. http://www.oardc.ohio-state.educ/seedid.

Coutts, B.A. and Jones, R.A.C.2015. *Potato virus Y*: Contact transmission, stability, inactivation and infection sources. *Plant Diseases* 99 (3): 387-394.

Cuevas, J.M., Delaunay, A., Bellstedt, D.U., Jaquot, E and Elena, S.F .2012. Phylogeography and molecular evolution of *Potato virus Y*. *Public Library of Science* **7**:37:38.

Desai, B. B., Kotecha, P. M. and Salunkhe, D. K. 1997. Seeds Handbook: Biology, Production, Processing and Storage, Marcel Dekker Inc., New York.

Dezfuli, P.M., Sharif-Zadeh, F. and Janmohammadi, M.2008. Influence of priming techniques on seed germination behavior of maize inbred lines (*Zea mays L*). *ARPN Journal of Agricultural and Biological Science* 3:22-25.

Drescher, A.N., Pasguini, M.W. and Shackleton, C.M.2009. African Indigenous in Urban Agriculture. ISBN 9781136574986.

Ekhuya, N.A., Wesonga, J.M. and Abukutsa, M.O.2018. Production, processing and storage techniques of African nightshade (*solanum spp*.) seeds and their correlations with farmers' characteristics in western Kenya. *African Journal of Food, Agriculture, Nutrition and Development*. 18(2):38-51.

Elizabeth, S. and Adeniji, O.T. 2015. Contract production of indigenous vegetable seeds; a case of East and Southern Africa. *African J. Agric. Res.* 3:303–309.

FAO, 2010. State of food insecurity in the World. Food and Agriculture Organization of the United Nations, Rome, Italy. http://www.fao.org/docrep/013/i1683e/i1683e.pdf.

FAO, 2013.The state of Food and Agriculture: Food systems for better nutrition. United Nations, Rome,Italy. Food and Agriculture Organization of the United Nations Rome, plant production and protection 202pp.

FAO/UNESCO, 2000. Revised legend for the soil map of Kenya. World soil resources report 68pp. Rome

Farooq, M., Basra, S. M. A., Hafeez K. and Ahmad N. 2005. Thermal hardening: a new seed vigor enhancement tool in rice. *Acta Botanica Sinica* 47: 187-193.

Fredslund, J., Schauser, L., Madsen, L. H., Sandal, N. and Stougaard, J. 2005. Using a multiple alignment of related sequences to find primers for amplification of homologs. *Nucleic Acids Res.* 33:516–520.

Gaya, A.S., Masinde, P.W., Murakami, K.and Ojiewo, C.O.2007. Mutation breeding of African nightshade (*Solanum spp*.). Global Science Books.

Ghosh-Jerath, S., Singh, A., Magsumbol, M.S., Kamboj, P and Goldberg, G. 2016. Exploring the potential of indigenous foods to address hidden hunger: nutritive value of indigenous foods of Santhal tribal community of Jharkhand, India. *Journal of Hunger and Environmental Nutrition* 11(4): 548-568.

Gray, S., Whitworth, J., Huimin, X., Singh and Karasev, A.2013. The current state of *Potato virus Y* (PVY) affecting potato grown in North America. North American Plant Protection Organization (NAPPO) science and technology documents, 1:5

Gibbs, A. and Mackenzie A, 1997. A primer pair for amplifying part of the genome of all potyvirids by RT-PCR. *Journal of Virological Methods* 63: 9–16.

Groves, C., German, T., Dasgupta, R., Mueller, D. and Smith, D.L. 2016. Seed Transmission of *Soybean vein necrosis virus*: The First *Tospovirus* Implicated in Seed Transmission. *PLoS ONE* 11(1):42.

Groot, P.C., 2004. Challenges for the production of high quality organic seeds. Seed Testing International No. 127 April 2004. Plant Research International, Wageningen University and Research Centre.

Gutierrez, P.A., Alzate, J.F. and Marin, M.2013. Complete genome sequence of a novel potato virus S strain infecting *Solanum phureja* in Colombia. Arch Virol, 158(10), 2205-2208.

Hamin, L., Mohanto, D.C., Sarker, M.A. and Ali, M.A.2014. Effect of seed borne pathogens in germination of some vegetable seeds. *Journal of Phytopathology and Pest Management* 1:34-51.

Handiseni, M., Sibiya, J., Ogunlela, V and Koomen, I. 2008. Comparative study of the effect of different weed management strategies on disease severity and marketable yield of paprika [*Capsicum annuum* L.] in the smallholder farming sector of Zimbabwe. *J. Plant Prot. Res*.48 (1):107-117.

HO, T. and Tzanetakis, I.E.2014. Development of a virus detection and discovery pipeline using next generation sequencing. *Virology Journal* 471:54-60

Hull, R.2009. Comparative Plant Virology, 5th Edition, Academic press, London, UK.

Icishahayo, D. 2014. Management of common bean (*Phaseolus vulgaris*) angular leaf spot (*Pseudocercospora griseola*) using cultural practices and development of disease-weather models for prediction of the disease and host characteristics. PhD Thesis. University of Zimbabwe. Zimbabwe.

IFPRI, 2012. Global food policy report. International Food Policy Research Institute Washington DC 114.

Islam, M.S. 2006. Study on the seed health status of selected vegetable crops. Msc Thesis, Department of Plant Pathology, Bangladesh University, Mymensingh. pp.47

Ismael, J.H.S.2010. Isolation and identification of some fungi from certain solanaceous seeds in Sulaimania and Germain regions and their exudate effects on germination rate. *Agriculture Biology Journal of America* 1:615-619.

ISTA, 2014. International Rules for Seed Testing. *Seed Science Technology* 24: 39-42.

Jacquemond, M. 2012. *Cucumber mosaic virus*. *Advances of Virus Research* 84, 439–504. doi: 10.1016/B978-0-12-394314-9.00013-0

Jaetzold, R., Schmidt, H., Hornet, B and Shisanya, C. 2006. Farm Management Handbook of Kenya. Ministry of Agriculture, Kenya and German Agency Technical Cooperation team (CTZ). Vol II/C1.

Jones, R.A.C.2000. Determining 'threshold' levels for seed borne virus infection in seed stocks. *Virus Research* 17:171-183.

Juliane, L., Lucie, A.T., Bernard, M., Waceke, W. and Carmen, B.2015. A first survey on plant virus infections of African nightshade from small farms in Western Kenya. Conference paper: Plant 2030 status seminar, at Potsdam 2:123.

Karavina, C., Chihiya, J. and Tigere, T.A. 2008. Detection and identification of *Xanthomonas phaseoli* in common bean (*Phaseolus vulgaris L.)* seeds. *Journal of Sustainable Development in Africa.* 10(1): 105-119.

Kaur, B. 2010. Development and evaluation of methods for the detection of seed-borne fungi in rice. *International Journal of Educational Administration*. 2(2):123-130.

Kerlan, C., Nikolaeva, O.V., Hu, X., Meacham, T., Gray, S.M. and Karasev, A.V. 2011. Identification of the molecular make-up of the *Potato virus Y* strain PVY (Z): Genetic typing of PVY (Z)-NTN. *Phytopathology* 101:1052-1060.

Kimiywe, J., Waudo, J.and Mbithe, D.2006. Reducing hidden hunger and malnutrition through traditional foods, IPGRI newsletter for sub-Saharan Africa. Issue no.21

King, A.M.Q., Adams, M.J., Carstens, E.B and Lefkowitz, E.J.2012. Virus taxonomy: Classification and nomenclature of viruses. Ninth Report of the International Committee on Taxonomy of Viruses. Elsevier Academic Press, a Diego, EEUU.1327.

Kiremire, B., Musinguzi, E and Lukwago, F.2010. Effects of vegetables drying techniques on nutrient content: A case study of South Western Uganda. *African Journal for Agriculture, Nutrition and Development* 10 (5):2587-2600.

Kreuze, J.F., Perez, A., Untiveros, M., Quispe, D., Fuentes, S., Barker, I. and Simon, R.2009. Complete viral genome sequence and discovery of novel viruses by deep sequencing of small RNAs. A generic method for diagnosis, discovery and sequencing of viruses .*Virology* 388:1-7.

Kumar, S., Udaya, S.A. and Nayala, S.C., Lund.,O.S. and Prakash,H.S.2011.Detection of *Tobacco mosaic virus* and *Tomato mosaic virus* in pepper and tomato by multiplex RT-PCR *Letters in Applied Microbiology* 53:359-363.

Latif, M.A, Rahman, M.M., Islam M.R.and Nuruddin, M.M.2009. Survey of arthropod biodiversity in the brinjal field *Journal of Entomology 6* (1), 28-34

Lee, J.W, Lee, S.H. and Kim, K. 2011. Development of RT-PCR based method for detecting five non-reported quarantine plant viruses infecting the family Cucurbitaceae or Solanaceae. *Plant Pathol. J.* 27(1):93-97.

Leslie,J.F and Summerell, B.A.2006.The Fusarium laboratory manual. Blackwell publishing, Hoboken Vol. 2.

Lievens, B. and Thomma, B.P.H.I.2005. Recent developments in pathogen detection assays: implication for fungal plant pathogens and use in practice. *Phytopathology* 95:1374-80

Liu, L., Xie, J., Cheng, J., Fu, Y., Li, G. and Yi, X. 2014. Fungal negative-stranded RNA virus that is related to bornavirus and nyavirus. *Proceedings of Natural sciences Academies U.S.A.* 111:205–210.

Madhubala, R., Bhadramurthy, V., Bhat, A.I., Hareesh, P.S., Retheesh, S.T and Bhai, R.S. 2005: Occurrence of *Cucumber mosaic virus* on vanilla (*Vanilla planifolia*) in *India Journal of Biosciences* 30:339–50.

Manoko, M.L.K.2007. A Systematic Study of African *Solanum L.* Section *Solanum (Solanaceae)* (Doctoral dissertation, Ph.D. thesis: Radboud Univesity Nijmegen).

Matasyoh, L.G and Nyang'au, A.B.2016. The *Solanum nigrum* complex (Black nightshade) grown in the Rift valley, Western and Nyanza provinces of Kenya. *Journal of life sciences* 10:228-232.

Matthews, S., Noli, E., Demir, I., Khajeh, H.M. and Wagner, M.H. 2012. Evaluation of seed quality: from physiology to international standardization. *Seed Science Research* 22:69-73.

Mathur, S.B and Kongsdal, D. 2003. Common laboratory seed health testing method for detecting fungi. First edition. International seed testing association. Bassersdorf, Switzerland. 425 pp.

Mathur, S.B and Olga, K.2001. Common Laboratory Seed Health Testing Methods of Detecting Fungi. Danish Government Institute of Seed Pathology for Developing countries. *Kandrups Bogtrigkkeri,* 1st edition. 411-413.

Mcdonald, M.B. and Kwon, F.Y.2004.Flower seeds. Biology and technology. CABI publishers, Cambridge, MA.

Meseret, D.R., Ali, M., Bantte, K. 2012. Evaluation of Tomato (*Lycopersicon esculentum Mill.*) Genotypes for Yield and Yield Components. *The African Journal of Plant Science and Biotechnology* 6 (1): 45-49.

Milošević, M., 2010. Vigour tests as indicators of seed viability. *Genetika* 42:103-118.

Moodley, V., Ibaba, J.D., Naidoo, R. and Gubba, A.2014. Full-genome analyses of a potato virus Y (PVY) isolate infecting pepper (*Capsicum annum* L) in the republic of South Africa. *Virus Genes* 49:466-76.

Mumbi, K., Karanja, N., Njenga, M., Kamore, M., Achieng, C.and Ngeli, P.2006. Investigative market research: Viable market opportunities and threats for urban and peri-urban farmers, Farm concern International, Urban harvest and international potato centre, Nairobi.

Musyimi, D.M. and Muthoni, J.2009. Growth responses of African nightshades (*Solanum scabrum*) seedlings to water deficit. *Journal of Agricultural and Biological Science* 5:8-16

Muthii, T.K.2014. Quality status of farm saved bean seed in Maragua and management of seed borne diseases by seed treatment. MSc Thesis. University of Nairobi, Kenya.

Mutuku, J.M., Wamonje, F.O., Mukeshimana, G., Njuguna, J.,Wamalwa, M., Choi, S.K., Tungadi, T.,Djikeng, A., Kelly, K., Domelevo, E. J.B., Ghimire, S.R., Mignouna, H.D., Carr, J.P. and Harvey, J.J.W. 2018. Metagenomic analysis of plant Virus. Occurrence in Common Bean (*Phaseolus vulgaris*) in Central Kenya. *Frontiers of Microbiol*. 9:29

Mwai, G.N., Onyango, J.C. and Abukutsa-Onyango, M.O.2007. Taxonomic identification and characterization of Africa nightshade (*Solanum* L. section *Solanum*). *Journal of food, Agriculture, Nutrition and Development,* Maseno University, Kenya.7:4.

Naidu, R. A and Hughes, J.D.A.2003.Methods for the detection of plant virus diseases. *Plant Virology in Sub-Saharan Africa* 4:233-253.

Naseri, B and Mousavi, S.S. 2015. Root rot pathogens in field soil, roots and seeds in relation to common bean (*Phaseolus vulgaris*), disease and seed production. *International Journal of Pest Management* 61(1):60-67.

NCBI, 2018.Database Resources of the National Center for biotechnology information on Genomes. http://www.ncbi.nlm.nih.gov/home/literature.shtml

Ngadze, E.2014. *In vitro* and greenhouse evaluation of botanical extracts for antifungal activity against *Phytophthora infestans Journal Biopesticides* 7(2):198-203.

Nyarango, R.M., Aloo, P.A., Kabiru, E.W and Nyanchongi, B.O.2008. "The Risk of Pathogenic Intestinal Parasite Infections in Kisii Municipality, Kenya." *BMC Public Health 8* (1): 237.

Ogunlela, Y. I.and Mukhtar, A. A. (2009). Gender issues in agriculture and rural development in Nigeria: The role of women. *Humanities and Social Sciences Journal,* 4:19-30.

Oiye, S., Shiundu, K. and Oniang'o, R. 2009. The contribution of African leafy vegetables (ALVs) to vitamin A intake and the influence of income in Rural Kenya, *African Journal Food Agriculture Nutrition Development* 10:6-8

Ojiewo, C.O., Mwai, G.N., Abukutsa-Onyango, M.O., Agong, S.G and Nono-Womdim, R.2013.Exploiting the genetic diversity of vegetable African nightshades. *Bioremediation, Biodiversity and Bioavailability*. 7(1):6-13.

Olayemi, F.F., Adegbola, J.A., Bamishaiye, E.I and Awayu, E.F.2012. Assessment of postharvest losses of selected crops in Rivers state Nigeria. *Asian Journal of Rural Development* 2(1):13-23.

Olet, E.A., Manfred, H. and Kare, A.2005. African Crop or Poisonous Nightshade; the Enigma of Poisonous or Edible Black Nightshade Solved. *African Journal of Ecology* 43:158-161.

Onim, M. and Mwaniki, P.2008. Cataloguing and evaluation of available community/farmers-based seed enterprises on African indigenous vegetables (AIVs) four ECA countries. Lagrotech consultants.

Opole, R.A, Mathenge, P.W., Auma, E.O., Van Rheenen, H.A.and Almekinders, C.J. 2003. On-farm seed production practices of common bean (*Phaseolus vulgaris L.) African Crop Science Conference Proceedings*. 6: 722-725.

Osborn, T., Napolitano, G. and Fajardo, J.2010. Seeds in Emergencies: A technical handbook. *Phaseoli* in common bean (*Phaseolus vulgaris L.)* seeds. *Journal of Sustainable Development in Africa* 10 (1): 105-119.

Owolabi, A.T. and Taiwo, M.A.2001.Effect of Celosia mosaic virus on *Celosia argentea* L. *Global Journal of pure and applied sciences* 7 (2):209-212.

Pandey, R. K. 2008. Plant Pathology. S. Chand and Company Ltd., New Delhi, India, 30 pp

Paylan, I.C. and Gumus, M.2013.Detection of viruses in seed of some vegetables by reverse transcriptase polymerase chain reaction (RT-PCR). *African Journal of Biotechnology* 12 (25):3891-3897.

Pryor, B.M. and Gilbertson, 2001. A PCR-based assay for detection of *Alternaria radiana* on carrot seed. *Plant diseases* 85:18-23.

Rabie, M., Ratti, C., Calassanzio, M., Aleem, E. A., and Fattouh, F. A. 2017. Phylogeny of Egyptian isolates of *Cucumber mosaic virus* (CMV) and *Tomato mosaic virus* (ToMV) infecting *Solanum lycopersicum*. *European Journal of Plant Pathology* 149: 219–225.

Robert, Y., Woodford, J.T and Ducray-Bourdin, D.G 2003. Some epidemiology approaches to the control of aphid-borne virus diseases in seed potato crops in northern Europe. *Virus Research* 71(1): 33-47.

Roossinck, M. J., Martin, D. P., and Roumagnac, P. 2015. Plant virus metagenomics: advances in virus discovery. *Phytopathology* 105:716–727.

Sabry, M. Y., Hosseny, M.H., El-Shaikh, K.A., Obiadalla, A.H.and Mohamed, Y.A. 2013. Seed borne fungal pathogens associated with common bean (*Phaseolus vulgaris L.*) seeds and their impact on germination. *Journal of Environmental Studies* 11:19-26.

Santorum, M., 2013. Comparison of tests for the analysis of vigor and viability in soybean seeds and their relationship to field emergence. *Acta Scientiarum Agronomy* 35 (1):83-92.

SAS., 2002. SAS Online Doc, Version 9.2. SAS Institute Inc., Cary NC., USA.

Sastry, K.S.2013. Diagnosis and detection of plant virus and viroid diseases in plant virus and viroid diseases in the tropics. Springer Netherlands. 353pp.

Schaad N.W., Jones J.B.and Chun W., 2001. Laboratory Guide for Identification of Plant Pathogenic Bacteria. 3nd. Ed. APS Press, St. Paul, MN, USA.

Schmidt, L.2000. Seed collection guide to handling of tropical and subtropical forest seed, http://en.sl.Life.ku.dk

Sharma, P.N., Sharma, O.P., Padder, B.A. and Kapil, R. 2008. Yield loss assessment in beans due to anthracnose (*Colletotrichum lindemuthianum*) under Sub Temperate conditions of North-Western Himalayas. *Indian Phytopathology* 61(3): 323-330.

Singh, R.P.,Valkonen, J.P.,Gray, S.M.,Boonham, N.,Jones, R.A.,Kerlan, C. and Schubert, J.2008,Discussion paper. The naming of potato virus Y strains infecting potato. *Archives of Virol*ogy 153:1-13.

Spence, N.J., Scaly, I., Mills, P.R. and Foster, G.D.2001. Characterization of a *Tobamovirus* from Trailing Petunias. *European Journal of plant pathology* 107:633-638.

Stefano, J. and Musya, D.G.2010. Responses of Maize (*Zea mays*) seed germination capacity and vigour to seed selection based on size of cob and selective threshing. *World Journal of Agricultural Sciences* 6(6):683-688.

Sthapit, B., Ram, R., Pashupati, C., Bimal, B. and Pratap, S. 2008. Informal seed systems and on-farm conservation of local varieties. In: Farmers, seeds and varieties: supporting informal seed supply in Ethiopia. *Wageningen International Journal* 2:7-10.

Svobada, J., Cervena, G. and Rodova, J.2006.First report of pepper mild mottle virus in pepper seeds produced in Czech republic. *Plant protection science* 42:34-37.

Taiwo, M.A.and Owolabi, A.T.2004. Occurrence and prevalence of viruses of leafy vegetables in Lagos Nigeria. *Global Journal of pure and applied sciences* 10 (1):61-67.

Taylor, J. 2014.The international rules seed testing: 2014 edition ISTA ordinary general meeting; Antalya Turkey.

Thompson, J. R., Langenhan, J. L., Fuchs, M., and Perry, K. L. 2015. Genotyping of Cucumber mosaic virus isolates in western New York State during epidemic years: Characterization of an emergent plant virus population. *Virus Research* 210:169–177.

Tungadi, T., Groen, S. C., Murphy, A. M., Pate, A. E., Iqbal, J. and Bruce, T. J. A.2017. Cucumber mosaic virus and its 2b protein alter emission of host volatile organic compounds but not aphid vector settling in tobacco. *Virology Journal* 14:91.

Uma, V. and Weseley, E.G.2003. Seed-borne fungi of rice from south Tamil Nadu. *Journal of Academia and Industrial Research,* 1:612-614

Vaidehi, B. K. 2002. Seed mycoflora of sunflower - a perspective. *Frontiers in Microbiology, Biotechnology and Plant Pathology*, 23: 25-40.

Van Rensburg, W.S.J., Van Averbeke, W., Beletse, Y.G. and Slabbert, M.M.2012. Black nightshade *(Solanum retroflexum* Dun.); production guidelines for African leaf vegetables. Water research Commission, Pretoria.pp.31-32.

Vanist, H., Sharma, D. and Gupta A, 2013.A review on commonly used biochemical test for bacteria. *Innovative Journal of life sciences* 1(1):1-7.

Walcott, R.R.2003.Detection of seed borne pathogens. *Horttechnology* 13:40-47.

Wang, B., Ma, Y., Zhang, Z., Wu, Z., Wu, Y., Wang, Q. and Li, M.2011.Review. Potato viruses in China. *Crop protection Journal* 30 (9):1117-1123.

Wang, F., Qi S., Gao, Z., Akinyemi, I.A., Xu, D. and Zhou, B.2016. Complete genome sequence of tobacco virus1, a closterovirus from *Nicotiana tabacum*. *Archives of virology* 161:1087-90.

Ward, E., Foster, S., Fraaije, B. and McCartney, H.A.2004. Plant pathogen diagnostics: immunological and nucleic acid-based approaches. *Annals of Applied Biology* 145:1-16.

Weinberger and Lumpkin, 2007. Diversification into horticulture and poverty reduction: A research agenda. *World Development* 35:1464–1480.

Wilkinson, C.A., Jones, J.J., Reed, T.D. and Tolin, S.A.2006. Seed transmission of *Cucumber mosaic virus* in spinach. *Virology Journal* Virginia 87 (9): 924-31.

APPENDICES

Appendix 1: African nightshade survey data collection questionnaire

Questionnaire no._____

A. QUESTIONNAIRE FOR FARMERS PRODUCING SEEDS

Date of interview: ..... ……..
Name of interviewer: .....
Name of Respondent: ..... ……
Occupation of Respondent: .....
County: ..... ……

AEZ: .....
Town ……
Village ……..
P.O. Box..... Phone: .....

Interviewer: (Introduce yourself and explain the purpose of this survey, which is to understand the production, quality status and processing of African nightshade. The farmers need to know that all information solicited will solely be used for research purposes).

A: Respondent's Background Information: (Please tick as appropriate)

1. Gender of household head/respondent: Male ( ) Female ( )
2. Age of household/responded (in years)<18 ( ) 19-30 ( ) 31-40 ( ) 41-50 ( ) 51-60 ( ) Over 60 ( )
3. Household Population size (insert number) Male ( ) Female ( )
4. Respondent's marital status Married ( ) Single ( ) Window ( ) Separated ( )
5. Type of land ownership 1. Ancestral ( ) 2. Bought ( ) 3. Rented ( ) 4. Others (please specify) ( )
6. Size of household farm (in acres)
   < 2 ( ) 2-4 ( ) 5-7 ( ) 8-10 ( ) 11-13 ( ) 14-16 ( ) 17-19 ( ) >20 ( )
7. Highest level of formal education

a. Not attended formal education ( )
b. Primary school ( )
c. Secondary school ( )
d. College University ( )

8. Household head main occupation.
   a. Agriculture and livestock ( )
   b. Business ( )
   c. Formal employment ( )
   d. Informal employment ( )
9. Spouse's main occupation.
   a) Agriculture and livestock ( )
   b) Business ( )
   c) Formal employment ( )
   d) Informal employment ( )
   e) Other (specify) …….

B: African nightshade production information

1. Based on morphological identifiers which African nightshade species is cultivated by the farmer.
   Solanum scabrum ( ) *Solanum villosum* ( ) Both ( )

2. How do you improve the fertility of the land
   a. Application of organic manures ( )
   b. application of inorganic fertilizers ( )
   c. application of both organic and inorganic fertilizers ( )
   d. no fertilizer application n ( )
3. In your own opinion is the farmer knowledgeable about the pathogens that infests African nightshade in the area Yes ( ) b. No ( )
4. If **Yes** which are the common pathogens that infest African nightshades (corroborate the farmers responses with field observations) ……

5. What are the common symptoms? (corroborate the farmers responses with field observations)
6. a. Would you attribute any of the above symptoms to a particular pathogen?Yes/No ……
   b. If **Yes** give a reason
7. a. Would you attribute any of the above symptoms to a particular pest?Yes/No ……
   b. If **YES** give a reason
8. Apart from African nightshade, what crops did you grow on your farm in the last 12 months? …….
9. What is the size of your land under African nightshade?
   a. Kitchen garden (less than 1/4acre) ( ) ¼ acre ( ) ½ acre ( ) ¾ acre ( )
   b. More than ¾ acre ( )
10. Why do you grow the African nightshade
    a. For home consumption ( );
    b. For income ( );
    c. Both for home consumption and income ( )
    d. Medicinal ( )
    e. Others (specify)
       …….
11. Where do you get seeds to grow African Nightshade?
    a. Market ( ) b. farm saved ( ) c. agrovet ( )
    d. Seed companies ( ) e. Government ( )
12. Which seed materials gives you better yields
    a. Market ( ) b. farm saved ( ) c. agro vet ( )
    d. Seed companies ( ) e. Government ( )
13. In what form/package is the seed material presented to you
    ……
    ……
14. What problems do you face in the production of African nightshade?
       ……
       ……

(This part should be administered to farmers who process own seeds)

15. a. Which method due you use when processing seeds
    a. Dry ( ) b. Wet ( )
    b. Briefly describe the procedure of processing
    ……
    ……
16. Why do you prefer the method identified above
    ……
    ……
17. List the equipment's used in processing the seeds
    ……
18. How do you maintain the purity of seeds during processing
    ……
19. In your own opinion is the processing method identified above carried out properly
    ……
20. What are the limitations of the identified processing method?
    ……

21. How do you dry seeds during processing?……
22. What costs due you incur in processing ANS seeds?
……
23. How long does it take to process the seeds?……
24. How do you select seeds for market?……
25. Does the following affect the seeds during processing?(tick where appropriate)
    a. Molds (YES/NO) b. Premature sprouting (YES/NO)
26. What other challenges due you face while processing the seeds
……
27. What impurities due you find in the farmers seeds
……
28. Comment on the germination percentage of the farm saved seeds
……
29. Do you use preservatives during seed processing
……
30. How much income do you get in selling the African nightshade to the market
……..... .....
31. How long do you store seeds before losing viability.....

B.QUESTIONNAIRE FOR SEED TRADERS/STOCKISTS.

Date of interview: ..... ……..
Name of interviewer: .....
Name of Respondent: ..... ……
Occupation of Respondent: .....
County: ..... ……
AEZ: .....
Town ……
P.O. Box..... Phone: .....

Interviewer: (Introduce yourself and explain the purpose of this survey, which is to understand the production, quality status and processing of African nightshade. The seed trader needs to know that all information solicited will solely be used for research purposes).

1. When did you start this business of selling seeds of indigenous vegetable?.....
2. Why do you sell African nightshade seeds and which type do you sell?
    a. ……
    b. ……
3. Where do you buy these African nightshade seeds?
   (i).....(ii).....
   (iii)..... (iv).....
4. Who produces these seeds? (i) Individual Farmers ( ) (ii) Farmer Groups ( ) (iii) CBOs ( ) (iv) NGOs ( ) (v) Seed Companies ( ) (vi) University ( ) (vi) Government Research Organization ( ) (vii) Government Extension ( ) (ix) Other.....
5. Do you buy these seeds/kg or grams? Kg Y/N .....grams Y/N.....
6. If YES, how much do you pay/kg? Local currency.....US$.....How about for say packs of 50 grams? Local currency.....US$.....
7. If NO, what measure is used? .....

8. What criteria do you use to select seeds for sale?
9. Do you consider the physical purity when buying the seeds Y/N
10. If yes what physical purity parameters do you consider
11. How much seed of each African nightshade do you buy/year?
    ……..
12. Do you have enough African nightshade seed supplies to meet farmers' demand? Y/N.....
13. If NO, what are the problems?.....
14. What prices do you charge for the seeds of various African nightshades?
    Package (g).....Price: Local currency.....US$.....
15. Who are the major buyers of African nightshade seeds?
    i. Men.....Women.....
       Boys.....Girls.....
16. During which months do you sell most of the African nightshade seeds?
    .....
17. Are your buyers happy with the seeds they buy from you? Y/N.....
18. IF YES, what are they happy about? .
19. IF NO, what are they complaining about?
20. What African nightshade do farmers like more?
    i. Their local varieties? Y/N.....
    ii. Improved varieties? Y/N.....
21. As an African nightshade seeds seller, what challenges do you face in seed supplies?
22. Between local varieties of African nightshades and improved types, which ones would you like to buy more for selling?
    i. Local unimproved varieties.....
    ii. Improved varieties.....

(This part should be administered to traders who process own seeds)

23 a. Which method due you use when processing seeds
   a. Dry ( ) b. Wet ( )
   b. Briefly describe the procedure of processing

……
25. List the equipment's used in processing the seeds
26. How do you maintain the purity of seeds during processing?
27. In your own opinion is the processing method identified above carried out properly
28. What are the limitations of the identified processing method?……
29. How do you dry seeds during processing?……
30. What costs due you incur in processing ANS seeds?……
31. How long does it take to process the seeds?……
32. How do you select seeds for market?……
33. Does the following affect the seeds during processing?(tick where appropriate)
    a. Molds (YES/NO) b. Premature sprouting (YES/NO)
34. What other challenges due you face while processing the seeds
    ……
35. What impurities due you find in the farmers seeds……
36. Comment on the germination percentage of the farm saved seeds……
37. Do you use preservatives during seed processing……
38. How much income do you get on selling the African nightshade to the market on monthly basis……
39. How long do you store seeds before losing viability.....

C.QUESTIONNAIRE FOR SEED COMPANIES.

Date of interview: ..... ……..
Name of interviewer: .....
Name of Respondent: ..... ……
Occupation of Respondent: .....
County: ..... ……
AEZ: .....
Town ……
P.O. Box..... Phone: .....

Interviewer: (Introduce yourself and explain the purpose of this survey, which is to understand the production, quality status and processing of African nightshade. The company personnel need to know that all information solicited will solely be used for research purposes).

1. When did you start this business of selling seeds of indigenous vegetable? .....
2. Who produces these seeds? (i) Individual Farmers ( ) (ii) Farmer Groups ( ) (iii) CBOs ( ) (iv) NGOs ( ) (v) Seed Companies ( ) (vi) University ( ) (vi) Government Research Organization ( ) (vii) Government Extension ( ) (ix) Other.....
3. Do you buy these seeds/kg or grams? Kg Y/N .....grams Y/N.....
4. If YES, how much do you pay/kg? Local currency.....US$.....How about for say packs of 50 grams? Local currency.....US$.....
5. If NO, what measure is used? .....
6. What criteria do you use to select seeds for sale?
7. Do you consider the physical purity when buying the seeds Y/N
8. If yes what physical purity parameters do you consider
9. How much seed of each African nightshade do you buy/year? ……..
10. Do you have enough African nightshade seed supplies to meet farmers' demand? Y/N.....
11. If NO, what are the problems?.....
    (ii) What prices do you charge for the seeds of various African nightshades?
    a. Package (g).....Price: Local currency.....US$.....
12. Who are the major buyers of African nightshade seeds?
    i. Men.....Women.....
       Boys.....Girls.....
13. During which months do you sell most of the African nightshade seeds? .....
14. Are your buyers happy with the seeds they buy from you? Y/N.....
15. IF YES, what are they happy about? .....
16. IF NO, what are they complaining about? .....
17. What African nightshade do stockists like more?
    i. Their local varieties? Y/N.....
    ii. Improved varieties? Y/N.....
18. What challenges do you face in seed supplies?
19. Between local varieties of African nightshades and improved types, which ones would you like to buy more for selling?
    i. Local unimproved varieties.....

ii. Improved varieties.....

20. Do you have any knowledge of farmer saved seeds(Y/N)
21. How do you compare the quality of cerfied seed and farmer saved seed?
22. Are you knowledgeable on how ANS seeds are processed (Y/N?)
23. Briefly explain how?
24. What challenges do you face in seed production
25. How long do your stock last and what is the frequency of restocking in a year How long do seeds take before losing viability?
26. What is the germination percentage of the seeds that you sell?
27. What is the purity percentage of the ANS seeds that you sell?.
28. Have stockists ever complained about non/poor germination on the seeds you once sold to them (Y/N)
29. If yes what was the nature of the complain
30. What were your sources of initial ANS foundation seed?
    i. International Research Organization Y/N,, if YES, which one
    ii. National Research Organization Y/N, if YES, which ones?
    iii. Government Extension Services Y/N (iii) University Y/N, if YES, which one?.....
    iv. NGO, Y/N, if YES, which one .....
    v. CBO, Y/N, if YES, which one .....
    vi. Religious Organization, Y/N, if YES, which one .....
    vii. Neighbor, Y/N. .....
    viii. Seed Company, Y/N, if YES, which one .....
    ix. Seed Stockist's shop, Y/N, if YES, which one .....
    x. Local seed seller in the market, Y/N .....
31. Do you experience difficulties getting ANS seeds for planting?
32. If YES, how have you solved the problem?
33. What is the quantity of ANS seeds imported into this country/year?.....mt?
34. Does this country export any ANS seeds? Y/N
35. Does the law allow for marketing of ANS standard seeds Y/N
36. Can you explain what you mean by standard seeds .....
37. Do we have Seed Laws and Regulations enacted to govern seed cerfication on ANS seeds?.....
38. Is it possible to get a copy of your Seed Laws and Regulations?Y/N
39. Does this country have a seed laboratory that is recognized by ISTA?Y/N

D. QUESTIONNAIRE FOR SEED CERTIFICATION AGENCIES.

Date of interview: ..... ……..
Name of interviewer: .....
Name of Respondent: ..... ……
Occupation of Respondent: .....
County: ..... ……
AEZ: .....
Town ……
P.O. Box..... Phone: .....

Interviewer: (Introduce yourself and explain the purpose of this survey, which is to understand the production, quality status and processing of African nightshade. The officer needs to know that all information solicited will solely be used for research purposes).

1. Are African nightshade (ANS) required to have mandatory seed certification in this country? Y/N
2. If NO, are there other requirements? Y/N
3. If YES, which are these requirements?
4. Which Seed Companies/Organizations grew these ANS seeds?
5. Are there any constraints to producing farmers or Seed Companies in production of certifiable ANS seeds? Y/N
6. If YES, which ones are these?
7. What purity parameters do follow in seed certification
8. Would field and seed inspections/ha of ANS cost the same?Y/N
9. If NO, would ANS cost less or more? Less , more
10. What quantities of ANS seeds are imported into this country?.....mt/year.
11. Does the law allow for marketing of ANs standard seeds Y/N
12. Can you explain what you mean by standard seeds .....
13. Do you have Quality Assured Seeds for ANS in this country? Y/N
14. How should seed be processed to get quality seeds
15. During seed testing do you get:
    i. Impure seed (Y/N)
    ii. Shriveled seeds (Y/N)
    iii. Discolored seeds (Y/N)
    iv. Other seed materials (Y/N)
    v. infected seeds (Y/N)
16. Do we have Seed Laws and Regulations enacted to govern ANS seed certification?
17. Is it possible to get a copy of your Seed Laws and Regulations? Y/N
18. Does this country have a seed laboratory that is recognized by ISTA? Y/N

Appendix 2: Mean composition per 100 gram edible portion of selected indigenous leafy vegetables compared to cabbage

Crop species	Amaranth	Spider plant	Black nightshade	Jute mallow	Cowpeas	Cabbage
Moisture content(g)	84	86.6	87.2	80.4	89.8	91.4
Iron(mg)	8.9	6.0	1.0	7.2	39	0.7
Protein(g)	4.6	4.8	43	4.5	4.6	1.7
Carbohydrates(g)	8.2	5.2	5.7	12.4	4.8	6.0
Fibre (g)	1.8	1.4	1.3	2.0	1.1	1.2

Ascorbic acid-vit c (mg)	64	13	20	80	87	54
Calcium	410	288	442	360	152	47
Phosphorus	103	111	75	122	120	40
B-carotene (microgram)	5716	10452	3660	6410	5700	100
Thiamine(Vit.B1) mg	0.05			0.15	0.35	0.04
Riboflavin(vit.B2) mg	0.42		0.59	0.53	0.2	0.1

Source: Grubben *et al.* (2004); (KENRIC), National Museums of Kenya; Maundu *et al.* (1999); Onyango, (2001)

Appendix 3: Chemical concentrations of Elisa reagents for detection of viruses

Washing buffer	1 liter of distilled water; 8.0g NaCl,0.2g KH_2PO_4,1.15g of Na_2HPO_4,0.2g NaN_3;0.20g of KCL L-containing 0.05% Tween -20,pH 7.4.
Conjugate buffer	1 liter of distilled water; 8.0g of NaCl, 0.2g KH_2PO_4, 1.15g of Na_2HPO_4, 0.2g of KCl, 0.20g of NaN_3, pH 7.4, 2% PVP, 0.2% egg albumin.
Substrate	0.02g of p-Nitrophenyl phosphate; 97ml of Diethanolamine; 600ml of distilled water; 0.20g sodium azide, make upto 1 liter, pH 9.8.

Appendix 4: *Potato virus Y* (PVY) aligned sequence used for high homology search in the NCBI database

>Seq1 [Organism=Potato Virus Y][RNA VIRUS INFECTING NIGHTHSHADE IN KENYA][mRNA,partial CDS

AKKCMATGRARRGAKAMSACCMACTYYATWRKGSTTWATGGCMRAMYCCMACTCMTCMT
TARCAAACMAAATTARKTGAAACMCSCTARATGGTTTCMAAATATGATACCCCRKTGGTTG
GGARCCMAACSARKCMACCMCATGGGATGGCTGCCTTTCSKGATCCACAATGGTCATTAGC
AAACAAAATAAGTTGAGACACGCTAGATGCTTTCAAAATATGATACCCAGTTGTTTGTGAGC
CAAACGAGTCAACCACATGGCATGTCTGCGTTTCGTGATCGACATCGGGCAACCCGATAGTG
TTATGTCAGACTCCACTCTCTTAGCAACTAAATAAGTTGAGACCGCTAGATGGAACAAAATA
TGATACCCAGTTGTTTGTGAGCCAAACGAGTCCMCCACATGGYATGTCTGCGTTTCGTGATC
RACASWMTCMGTGAGCGGATCAACGAACTGGATGAATGAATATTCTGTTGGGTCAAATCCA
TACATATTAACAAACCTCCTGCTTGACTTGCCCATACCAACAGTGGTGCCTTTACCTTTTCCC
TTCTTCCTGTATGCAGATCCAAAGAATTCCTCTATTGTGTCATCATTGTTGTCAATTTCAAAG
CCAGCCCTTTTGTCACGAGCATGGCGAAACTTCAAGGCTTGAATTCTTTTGGATTTATTTTTC
CCTTGGTGAGACACAGTCTCGACTGATTGTGTGAACCAACTATATATGAGTCCTATTCCACC
AATTGCAACAGCGCCTGCTATGATCAAGTCTTTGGCCACTAATGACTTCTTCCAAGTCCCCTT
CAACTTGAGATCCATATTTTGAAAGCATCTAGCGTGTCTCAACTTATTTTGTTTGCTAATGAT
GAGTTGGAGTCTGACATTAAGCACTATAGAGTTGGTGGTATTCCTAATGCATGCCCTGAACT
TGGGGTCACTTAACAGATTACAACAACTCTAAATGCTGTTGAAKACTCGAAMTCAGCTTAM
CAASAATTTAATAAGAGACACGCTMCCGCTTTCTRAAWGATACCCATTTATTTGTGAGCCAR
CMACCWCCRCAAGACCAGACTGCGTTTCCMGGCGCATGCAATTGGTGGAATAGGACTCATA
TATAGTTGGTTCACACAATCAGTCGAGACTGTGTCTCACCAAGGGAAAAATAAATCCAAAA
GAATTCAAGCCTTGAAGTTTCGCCATGCTCGTGACAAAAGGGCTGGCTTTGAAATTGACAAC
AATGATGACACAATAGAGGAATTCTTTGGATCTGCATACAGGAAGAAGGGAAAAGGTAAAG
GCACCACTGTTGGTATGGGCAAGTCAAGCAGGAGGTTTGTTAATATGTATGGATTTGACCCA
ACAGAATATTCATTCATCCAGTTCGTTGATCCGCTCACTGGAGCCCAAATTGAAGAGAACGT
CTATGCTGATATTAGAGACATCCAAGATCGCTTTAGTGATGTCCGCAAGACMTCCATTAACG
CCACCACCAACTCTATWGTGCTTAATGKCMRACTCCAACTCMTCMTTAACAAACMAAATAA
RTTGGGACMCSCTARATGCTTTCMAAATATGATACCCARTTGKTTGKGARCCMAACSARTCA
ACCMCATGGSATGKCTGCSTTTCSTGATCSACAATGAAATAAGTTGGGACACGCTAGATGCT
TTCAAAATATGATACCCAGTTGTTTGTGAGCCAAACGAGTCAACCACATGGCATGTCTGCGT
TTCGTGATCRACAAAAATAAGTTGGGACACGCTAGATGCTTTYAAAATATGATACCCAG

>Seq2 [Organism=Potato Virus Y][RNA VIRUS INFECTING NIGHTHSHADE IN KENYA][mRNA,partial CDS

ATATGAGTCCTATTCCACCAATTGCAACAGCGCCTGCTATGATCAAGTCTTTGGCCACTAAT
GACTTCTTCCAAGTCCCCTTCAACTTGAGATCCACTAGCGTGTCCCAACTTATTTTGTTTGCT
AATGATGAGTTGGAGTCTGACATTAAGCACTATAGAGTTGGTGGTATTCCTAATGCATGCCC
TGAACTTGGGATAGCAGGCGCTGTTGCAATTGGTGGAATAGGACTCATATATAGTTGGTTCA
CACAATCAGTTGAGACTGTGTCTCACCAAGGGAAAAATAAATCCAAAAGAATTCAAGCCTT
GAAGTTTCGCCATGCTCGTGACAAAAGGGCTGGCTTTGAAATTGACAACAATGATGACACA
ATAGAGGAATTCTTTGGATCTGCATACAGGAAGAAGGGGAAAGGTAAAGGCACCACTGTTG
GTATGGGTAAGTCAAGCAGGAGGTTTGTTAATATGTATGGATTTGACCCAACAGAATATTCA
TTCATCCAGTTCGTTGATCCGCTCACTGGAGCTCAAATTGAAGAGAACGTCTATGCTGATATT
AGAGACATCCAAGAGCGCTTTAGTGATGTCCGCAAGATAGCGTGTCCCAACTTATTTTGTTT
GCTAATGATGAGTTGGAGTCTGACATTAAGCACTATAGAGTTGGTGGTATTCCTAATGCATG
CCCTGAACTTGGGAGCCTCATCATTAGCAAACAAAATAAGTTGGGACACGCTAGATGCTTTC
AAAATATGATACCCAGTTGTCTGTGAGCCAAACGAGTCAACCACATGGCATGTCTGCGTTTC
GTGATCGACATGATGAGTTGGAGTCTGACATTAAGCACTATAGRGTTGKTGGTATTCCTAAT
GCATGCCCTGAACTTGGGAACCCCTCTAGMGTGTCCCAACTTATTTTGTTTGCTAATGATGA
KYTGGAGYCTGMCATTAAGCACTATMRAGTTGRWGSTATTCCTAATGCATGCCCTGAACTT
GRGATTCCCTTCTAGCGTGTCCCAACTTATTTTGTTTGCTAATGATGAGTTGGAGTCTGACAT
TAAGCACTATAGAGTTGGTGGTATTCCTAATGCATGCCCTGAACTTGGGYTGTTAATATGTA
TGGATTTGACCCAACAGAATATTCATTCATCCAGTTCGTTGATCCGCTCACTGGAGCTCAAAT
TGAAGAGAACGTCTATGCTGATATTAGAGACATCCAAGAGCGCTTTAGTGATGTCCGCAAGA
CCGTATTCATCCTCCTCTTCAAYGACTCAATTTTGGTTTAAGGAAAAAATACAAMTGGGRCT
CAMGAAAGCAGAAAAAAGAGACGACCCAAAAGTGTTTGAAAATGAAACCCSTTTGCTTGTG
GCACCWCTGCKTTTACCAAAAGGSCTGTCTCGGTTTATTATCTACAAGGGMAAAKRGYGCC
CCTTGTTGKCCCKTWTGGGGATTCTTTGGAACCGCMTRCCTGTTTGAKGGGAAAATGGYAA
GGCCCCCCTGGTGGATTGTTATTAAAAAATAGATGTCCTTTAAGAGAACCGGGATTCGCRCC
TAMMGAGAATCCCCCTATATARGGGGSGTATTCCCCCCCTGGGGCSCACAKCGCAAMAARG
CCYARGTSTTTATCTAAAAAAACCTTYACCGTTTTCTTTTGTTCTTGGAAAACAAATCATTAG
CAAACAAAATAAGTTGGGACACGCTAGATGCTTTCAAAATA

>Seq3 [Organism=Potato Virus Y][RNA VIRUS INFECTING NIGHTHSHADE IN KENYA][mRNA,partial CDS

GCCTGCTATGATCAAGTCTTTGGCCACTAATGACTTCTTCCAAGTCCCCTTCAACTAGCGTGT
CCCAACTTATTTTGTTTGCTAATGATGAGTTGGAGTCTGACATTAAGCACTATAGAGTTGGTG
GTATTCCTAATGCATGCCCTGAACTTGGGTTGATCATAGCAGGCGCTGTTGCAATTGGTGGA
ATAGGACTCATATATAGTTGGTTCACACAATCAGTTGAGACTGTGTCTCACCAAGGGAAAAA
TAAATCCAAAAGAATTCAAGCCTTGAAGTTTCGCCATGCTCGTGACAAAAGGGCTGGCTTTG
AAATTGACAACAATGATGACACAATAGAGGAATTCTTTGGATCTGCATACAGGAAGAAGGG
GAAAGGTAAAGGCACCACTGTTGGTATGGGTAAGTCAAGCAGGAGGTTTGTTAATATGTAT
GGATTTGACCCAACAGAATATTCATTCATCCAGTTCGTCGATCCGCTCACTGGAGCTCAAAT
TGAAGAGAACGTCTATGCTGATATTAGAGACATCCAAGAGCGCTTTAGTGATSCCTTTAAGG
GAACCCCCCAMTTCTTTAWTGTTWATGGYMAAATCCCACTTCTTCTTTACMAAMCAAATTA
AKTGGGAACCCCCTAAAGGTTTCCAAAATATAAACCCCATTGGTTGGGGACCCAACCAAKY
CACCCCCTGGGMTGGATGGCTTTTCKGAACCAAAAKGGATCATTAGCAAACAAAATAAGTT
GGGACACGCTAGATGCTTTCAAAATATGATACCCAGTTGTTTGTGAGCCAAACGAGTCAACC
ACATGGCATGTCTGCGTTTCGTGATCGACATTGGCAAKGCCATTCGTCTTGTCACAAACTTTG
AGTGGATTATGTGGCATTAAATCAATCTTCAAAGCTTTGTCAGACCAATCTTTCCTGAAGTAT
GCATGTATGGTCGTGTTACTGCCCAAGGCTTGCATTTCAATGTCATCATTCTCAACCATTTTC
TTTCGCACTTCACTAAATCTCTCTTGAATATCTCTAATGTCAGCATAGACATTCTCTTCTATTT
GCGCCCCAGTGAGTGGATCAACGAATTGGATGAATGAGTACTCTGTTGGATCAAACCCATAC
ATGTTGATGAACCTCCTGCTTGACTTGCCCATACCAACTGTGGTACCTTTACCTTTTCCCTTTT
TCCTGTATGCAGATCCAAAGAATTCCTCTATTGTGTCATCATTGTTGTCAATTTCAAAGCCAG
CCCTTTTGTCACGAGCATGGCGAAACTTCAAGGCTTGAATTCTTTTGGATTTATTTTTCCCTT
GGTGAGACACAGTCTCAACTGATTGTGTGAACCAACTATATATGAGTCCTATTCCACCAATT
GCAACAGCGCCTGCTATGATCAAGTCTTTGGCCACTAATGACTTCTTCCAAGTCCCCTTCAAC
TTGAGATCCCCCCAAAATAAATAAACATCTCCCATTCTTTTTTTMCAAACATATATAGKGTG
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GYGGTGTTCCCSTGTATGCCCGGACACAGGGKTCCCCGGGGTTGCGGGCCMTCCGCAAAAA
AACTAGCGTGTCCCAACTTATTTTGTTTGCTAATGATGAGTTGGAGTCTGACATTAAACATTA
TAGAGTTGGTGGTGTTCCTAATGCATGCCCTGAACTTGGGACACGATGGAGGTCCAACTCCC
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AGGCGCTGTTGCAATTGGTGGAATAGGACTCATATATAGTTGKTTCACACAATCAGTTGAGA
CTGTGTCTCACCAAGGGAAAAATAAATCCAAAAGAATTCAAGCCTTGAAGTTTCGCCATGM
TCGTGACAAAAGGGCTGGMTTTGAAATTGACAACAATGATGACACAATAGAGGAATTCTTT
GGATCTGCATACAGGAAAAAGGGAAAAGGTAAAGGTACCACAGTTGGTATGGGCAAGTCAA
GCAGGAGGTTCATCAACATGTATGGGTTTGATCCAACAGAGTACTCATTCATCCAATTCGTT
GATCCACTCACTGGGGCGCAAATAGAAGAGAATGTCTATGCTGACATTAGAGATATTCAAG
AGAGATTTAGTGAAGTGCGAAAGAAAATGGTTGAGAATGATGACATTGAAATGCAAGCCTT
GGGCAGTAACACGACCATACATGCATACTTCAGGAAAGATTGGTCTGACAAAGCTTTGAAG
AT

Appendix 5: *Cucumber mosaic virus* (CMV) aligned sequence used for high homology search in the NCBI database

>Seq1 [organism=*Cucumber mosaic virus* (CMV) Kenya in African Nightshade Kenya

AATCCCTGCCTCCCCTGTGAAATCAACCCTTGAAAACCTCTCTCTTCTAATCTTTTCTTTGTAATTCCTATGGCGACGT
CCTCGTTCAACATACAATGAACTGGTAGCCTCCCACGGCGATAAAGGACTACTCGCGACCGCCCTCGTTGATAAAA
CAGCTCATGAGCAGCTTGAGGAGCAATTACAACATCAACGTAGAGGCCGTAAGTCTACATTCGGAATGTTCTGAG
TGTAAGGATTCCGAGATCATTCGGAATCGGTATGGAGGGAAGTACGACCTCCATTACCCAGCAGGAGTTTGTCTC
CCCACGGCCTAGCTGGTGCCCTCCGCTTGTGTGAAACTCTCGATTGTCTAGACTCTTTCCCTTCTTCAGGTCTGCGG
CAGGACCTTGTCTTAGACTTCGGAGGAAGTTGGGTCACACATTACCTCCGCGGACACAACGTACACTGCTGTTCCC
CTTGTTTGGGTATCCGCGATAAAATGCGCCACGCGGAACGTTTAGTGAACATGCGCAAGATCATCTTGAACGATCC
ACAACAGTTCGATGGTCGGCAGCCGGACTTCTGCACTCAGCCCGCTGGCGGATTGCAAAGTACAAGCCCACTTTG
CTATATCCATTCATGGAGGTTATGATATGGGCTTCAGAGGATTAGTGAAGCGATGAATGCTCACGGGACCACTTTT
TTGAAGGG

GACGATGATGTTCGATGGTGCGATGATGTTTGACGACCAAGGCGTAATACCTGAACTTAACTGCCAGTGGAGGAA
GATTAGGAGTGCTTCTCCGAAACTGAAGACGTCACACCGGTGTCTGGTAAGCTTAATTCCACATTCTCTCATGTTCG
TAGGTTTAAGACTATGGTAGCTTTCGATTTTATCGACGAGTCTACCATGTCTTACGTTCATGATTGGGAGAATATAA
ATCTTTTCTTACCGACCAGACTTACTCTTACAAAGGAATGACCTATGGTATTGAACGTTTGTCATTAATGCCGGTATT
CATGACGTATAAGATCATTGGTGTGCCTGGGATGTGTCCACCCGAACTCATTCGACATTGTATTTGGTTCCCCTCGC
TTAAAGACTATGTTGGTCTGAAGATTCCCACGTCGCAGGATTGTTGAGTGGAAAACAGTGCGCATTTTAACGTCAA
CATTGCGTGAGACTGAAGAGATTGCTATGAGATGTTACAATGATAAGAAGGCGTTGGATGGAACAATTTAAGGTT
ATCCTAGGTGTTCTATCCGCTAAATCATCCACCATTGTTATCAATGGTATGTCCATGCAATCCGGTGAACGGATAGA
TATCAATGATTATCAACTTAATGAATGTCCGATTGTTAGTAGGGTTTGGTTGACGAAGTCGTGCTGCTACACTTTGG
TCAATTATGTGCCGTCATGTCTAAATTGAAGGCTGTGCGAGCTATATGTTTTGGAGATTCAGAACAAATTGCCTTCT
CTTCTCGAGATGCCTCGTTTGACATGCGTTTCTCGAGATTATTCCTGATGAGACTAGTGACGCGGATACCACATTCC
GTAGCCCACAAGACGTTGTGCCGCTTGTGCGTTTGATGGCTACGAAGGCCCTTCCGAAAGGAACCCGTTCAAAAT
ACACAAATGGGTTTCTCAATCTAAAGTGAAGAGATCTGTTACATCGCGTGTTATCGCTAGTTGACATTGGTTGACTT
GGATTCGACCAGGTTTTACATTACGATGACCCAAGCTGATAAGGCTTCACTGATTTCGAGGGCGAAAGAGATGAA
TCTCCCAAAGACTTTCTGGAATGAAAGGATAAAAACAGTACATGAGTCTCAAGGCATTTCTGAGACCATGTTACTT
TGGTGAGATTAAAGAGCACAAAGTGTGACCTGTTTAAACAATTTTCTTATTGTCTCGTTGCCTTGACTAGACATAAG
GTCACTTTCCGCTACGAGTATTGTGGTGTATTGAACGGCGATTAATCGCCGAATGTATTGCTCGTGCTTAGCGGTTT
CCCGCTTTTAGGCGGATCTGAGTTGGTAGTATATAACTGCCTGAGTCACTAAACGTGTTATACGGTGAACGGGTTG
TCCATCCAGCTTACGGCTAAATGGTCAGTCGTGGAGAAAT

Appendix 6: *Tobacco mosaic virus* (TMV) aligned sequence used for high homology search in the NCBI database

>Sequence 1 [organism Tobacco mosaic virus] [rRNA ANS isolate-Kenya complete genome

ATATGTCTTACAKTWTCACTACTCCATTCCRGTTCGTGTTCTTGTCATCAGCGTGGGCGACCCAATAGAGTTRTTAA
TTTATGTACTAATGCCTTAGGAAATCAGTTTCWWACACAACAAGCTCGAACTGTCGTTCAAAGACAATTCAGTGA
GGKGTGGAAACCTTCACCACAAGTAACTRTTAGGTTCCCTGACAGTGACTTTAAGGTGTACAGGTACAATGCGGTA
TTAGACCCGCTAGTCACAGCACTGTTAGGTGCATTCGACACTAGAAATAGAATAATAGAAGTTGAAAATCAGGCG
AACCCCACGACTGCCGAAACGTTAGATGCTACTCGTAGAGTAGACGACGCAACGGTGGCCATAAGGAGCGCGAT
AAATAATTTAATAGTAGAATTGATCAGAGGAACCGGATCTTATAATCGGAGCTCTTTCGAGAGCTCTTCTGGKTTG
GTCTGGACCTMAAAWCAYKTKATTTTTTWTTTTTTATTTTTAATWATTATTTWATTTTTTKTTTCATTTATTATTTTW
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CTAAAASATAAAAAAAAAAAAAAAATAAAACAAATAAAAARAAAAAAAAAAAAAGAAAAKAAAAAAAWAAAAAA
AAAAAAAATAAAAACAAGAAAAWAAATAAAAAAAAATAGACAAAAAAAAAGAGAAAAAAATAAAGAAARAAGA
AARAAAASACWSAATAATAGAACMARCRATACATRWAAGAAATTAAAAAAAATAAGARRMAAAAAAAAAYAKA
ATAAMMCAWAAR MRAAATCGGWWMAAKACAAWGACCATTAGATTAS

>Sequence 2

ATATGTCTTACAGTATCWCTACTCCATTCCAGTTCGTGTTCTTGTCATCAGCGWGGGCCGACCCAATAGAGTTAAT
TAATTTATGTACTAATGCCTTAGGAAATCAGTTTCWWACACAACAAGCTCGAACTGTCGTTCAAAGACAATTCAGT
GAGGTGTGAAACCTTCACCACAAGTAACTRTTAGGTTCCCTGACAGTGACTTTAAGGTGTACAGGTACAATGCGGT
ATTAGACYYGYTAGTCACAGCACTGTTAGGTGCATTCGACAYTAGAAATAGAATAATAGAAGTTGAAAATCAGGCG
AACCCCACGACTGCCGAAACGTTAGATGCTACTCGTAGAGTAGACGACGCAACGGTGGCCATAAGGAGCGCGAT
AAATAATTTAATAGTAGAATTGATCAGAGGAACCGGATCTTATAATCGGAGCTCTTTCGAGAGCTCTTCTGGKTTG
GTCTGGACCTMAWKTTCCTTYTGTTTTTTTKTTTTTTTTTTTTTTTTTTTTGTTTTGTTATCCTTTTTTTTTTTTTTTTTA
TKTTTTTTTTTTTTTTCTTTTTATTTATTAATTATGTTCCMAATTTAAYTTTKYAATATTTTATTTTTTKTCTGTTTTTTT
TGCTCCTTTATCTTAMTGARCTTGGCTSACTATWAATACTAAGATWATCCATTGMTCTAACTACCTCGAGAGAKCA
ATASCGAKWGAYAACGCACAARGAAACCAARAGAKAATAGRARYATMACCTACATAAAYRAAGAWAAAGAYWT
AGGTAWAACTGGMCATYCCAWAAKGGASAYASAAAAAYAMAAGARAAAAAAGAGAATAKAAAAKAATTWRA
AAWKAAATCAMATTATTATAKTTWTWTWWACAAYAWGAYACTAAAATAAGATAYTRAATAGAAATMATASTAK
AATAGRTAYTAMAATCTAGATAGAGTRAGAATATACASTAAGAGATGATAAGTATAAKCSAGYWGTYKGAYAACK
ACATASWAAGATAGTAATKACAKATASCAAATGYWTASRMTTASGAMARTTARWTWGCAAKRMTAAKKMT
GAKKAAGGAA WAMAWWRARKATCCAAATSSCGGCCSTTCCATTATTYATTKWCAAAC

Appendix 7: Analysis of variance for seed purity from different sources

Source of variation	df	Sum of squares	Mean of squares	F value	Pr >F
Replicates	3	6311.9	12.32927	0.05	0.080^{ns}
Treatment (seed sources)	3	1020.01	17.23456	11.023	0.0024*
Residual	21	3062.0			
Total	27	10393.91			

*significant at the p≤ 0.05 probability level.ns-Not significant at p≤ 0.05 probability level.

Appendix 8: Analysis of variance for germination parameters for seed quality determination

Source of variation	df	Sum of squares	Mean of squares	F value	Pr >F
Replicates	3	2532.4	32.6	2.31	0.3241^{ns}
Treatment (sites)	4	8634.2	17.2	4.89	0.00212*
Residual	35	2123.2	61.2		
Total	42	13289.8			

*significant at the p≤ 0.05 probability level.ns-Not significant at p≤ 0.05 probability level.

Appendix 9: Analysis of variance for detection of fungal pathogens in African nightshade

Source of variation	df	Sum of squares	Mean of squares	F value	Pr >F
Replicates	3	4251.7	162.4	0.09	0.0022^{ns}
Treatment (fungal)	7	2466.1	178.3	8.31	0.03242*
Residual	12	6223.4	104.1		
Total	22	12941.2			

*significant at the p≤ 0.05 probability level.ns-Not significant at p≤ 0.05 probability level.

Appendix 10: Analysis of variance for detection of bacterial pathogens in African nightshade

Source of variation	df	Sum of squares	Mean of squares	F value	Pr >F
Replicates	3	5212.8	23.4	0.82	0.0026*
Treatment (bacterial)	2	3233.1	68.2	6.42	0.00321*
Residual	10	5634.0	22.1		
Total	15	14079.9			

*significant at the p≤ 0.05 probability level.ns-Not significant at p≤ 0.05 probability level.

Appendix 11: Analysis of variance for detection of viral pathogens in African nightshade

Source of variation	df	Sum of squares	Mean of squares	F value	Pr >F
Replicates	3	7866	924.6	0.0712	0.0096*
Treatment (viral)	6	30224	152.4	18.22	0.00203*
Residual	100	26281	138.2		
Total	119	64371			

*significant at the p≤ 0.05 probability level.ns-Not significant at p≤ 0.05 probability level.

Appendix 12: Analysis of variance for African nightshade seed processing method

Source of variation	df	Sum of squares	Mean of squares	F value	Pr >F
Replicates	3	1067.4	43.72	3.43	0.0263^{ns}
Treatment (Method)	4	5334.66	28.28	8.6	0.01302*
Residual	28	6267.22	32.26		
Total	35	12669.28			

*significant at the p≤ 0.05 probability level. ns-Not significant at p≤ 0.05 probability level.

Appendix 13: Analysis of variance for seed germination parameters on seed processing

Source of variation	df	Sum of squares	Mean of squares	F value	Pr >F
Replicates	3	13002	46.56	0.62	0.0003^{ns}
Treatment (germination parameters)	4	20132	63.20	23.21	0.0062*
Residual	11	82245	38.11		
Total	18	115379			

*significant at the p≤ 0.05 probability level.ns-Not significant at p≤ 0.05 probability level.

Appendix 14: Analysis of variance for field parameters on seed processing methods

Source of variation	df	Sum of squares	Mean of squares	F value	Pr >F
Replicates	3	18762	34.12	0.081	0.0001*
Treatment (parameters)	4	9643.6	86.2	4.81	0.0062*
Residual	100	37652	34.60		
Total	107	66057.6			

*significant at the $p \leq 0.05$ probability level. Ns-not significant at $p \leq 0.05$ probability level.

Appendix 15: Survey site map of Kakamega and Kisii counties