HEALTH STATUS OF POTATO SEED AND HOST RESISTANCE AGAINST LATE BLIGHT DISEASE UNDER GREENHOUSE AND FIELD CONDITIONS IN KENYA

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DECLARATION

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

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To my husband Francis Jomo Kamuyu and our sons Michael Jomo and Charles Gitu Jomo

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LIST OF ACRONYMS AND ABBREVIATIONS

ANOVA	Analysis of Variance
AUDPC	Area Under Disease Progress Curve
AEZ	Agro Ecological Zone
BW	Bacterial wilt
BCIP	Toluidine salt of 5-bromo, 4-cloro, 3-indolyl
CIP	International Potato Centre
CABI	Centre for Agriculture and Biosciences International
DAS - ELISA	Double Antibody Sandwich-Enzyme Linked Immuno-Sorbent Assay
DMF/NBT	Dimethylformamide 70% in H_2O/ Nitro blue Tetrazolium
DMF/BCIP	100% Dimethylformamide / Toluidine salt of 5-bromo, 4-cloro, 3-indolyl
DAP	Diammonium phosphate
FAO	Food and Agriculture Organization
GIS	Geographical Information System
GPS	Geographical Positioning System
KALRO	Kenya Agricultural and Livestock Research Organization
KEPHIS	Kenya Plant Health Inspectorate Service
LH	Lower highland
MOA	Ministry of Agriculture
NBT	Nitro blue Tetrazolium
NCM	Nitrocellulose Membrane
NPK	Nitrogen phosphorous potassium
PVA	Potato Virus A

PVX	Potato Virus X
PYDV	Potato Yellow Dwarf Virus
PVY	Potato Virus Y
PLRV	Potato Leaf Roll Virus
TRV	Tobacco Rattle Virus
TSWV	Tomato Spotted Wilt Virus
TYLCV	Tomato Yellow Leaf Curl Virus
SMSA	Semi selective Media South Africa
SPSS	Statistical Package for the Social Sciences
UH	Upper highland

ABSTRACT

Importation of potato seed into Kenya has become common in light of the persistent shortage of clean seed potato in the country. The performance of imported potato varieties under Kenyan conditions and disease reaction especially to late blight caused by *Phytophthora infestans* is unknown. To fill this knowledge gap, a study was conducted on potato health status of local and imported germplasm, levels of resistance to late blight and yields under Kenyan conditions. A survey was carried out in Meru, Nyeri, Kiambu, Nakuru and Nyandarua counties to assess seed potato production systems and disease prevalence. The level of disease infection for imported and local potato seeds was determined. Evaluation of resistance to late blight and performance for twenty four varieties (local and imported) was conducted under greenhouse and field conditions at the University of Nairobi, KALRO's Genetic Resources Research Institute at Muguga and the Njabi-ini Sub-Centre of KALRO in Nyandarua County. A randomized complete block design with three replicates of each variety was used. Six local varieties and eighteen imported cultivars were planted. Arka variety was planted as a spreader row for late blight inoculum. The findings showed that area allocated to potato production in the surveyed counties varied with Nakuru having the highest average acreage of 0.7ha. Most of the farmers interviewed did not use certified seeds and therefore, incidences of bacterial wilt and potato leaf roll diseases were high. Nyeri County was leading with 90% bacterial wilt prevalence while Nyandarua had 91% potato leaf roll virus prevalence. Counties planting uncertified seeds had high incidence of bacterial wilt and viral diseases but, disease incidence was low in counties that planted certified seed indicating correlation between seed source and disease prevalence. The local (6) and imported (18) varieties showed variation in late blight susceptibility and resistance in both greenhouse and field studies. Under greenhouse conditions, all imported varieties had high late

blight intensity with area under disease progress curve (AUDPC) value ranging between 500 and 2250. Local varieties had AUDPC values between 20 and 46 with Sherekea having the lowest AUDPC value of 20 followed by Shangi, Asante, Nyayo, Kenya Mpya and Tigoni with the highest value of 46. Susceptibility and resistance to the late blight varied greatly also under field conditions. Most of the imported varieties were susceptible to late blight and the disease was more severe at Njabi-ini than at Muguga and Kabete sites. Carolus and Arnova (imported) recorded low (1.0) AUDPC values. In contrast, Kenya Mpya and Sherekea (local) showed significantly higher ($P<0.001$) resistance to the disease compared to all other varieties. The yields differed significantly ($P<0.001$) among varieties due to challenge of late blight disease at both greenhouse and field conditions. Cultivar Arnova had a mean yield of between 15.9 and 18.6 t/ha the highest being at University of Nairobi trial site with low rainfall and temperatures of between 20^0C and 22^0C. Carolus had a mean yield of between 12.2 and 19.5 t/ha and the highest was from Njabi-ini with high rainfall and low temperature of 18^0C and below. Carolus and Arnova had yields of over 36.6 t/ha at all the sites but, most of the other imported varieties had yields below average yields of 7.7 t/ha. Sherekea and Kenya Mpya yielded between 25.7 and 31.7 t/h respectively at Njabi-ini. Mean of Sherekea and Arnova had no significant difference indicating there was no influence of site interaction.

Potato seed from farmers' production system are contaminated with diseases and bacterial wilt is widely spread in Kenya. KALRO certified seed potato are free from *R. solanacearum* and viruses and they are produced in disease free sites. All the imported potato varieties are susceptible to late blight under greenhouse conditions and Carolus and Arnova are resistance to the disease under field conditions in Kenya. Carolus and Arnova varieties can be compared with Sherekea and Kenya Mpya in terms of resistance to late blight.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background information

Potato (*Solanum tuberosum*) is reported to have originated from the Andean highlands of South America (Razdan and Matto, 2005; Randall *et al*., 2010). The crop has been cultivated there for at least 8,000 years. Currently, potato is cultivated throughout the world. The crop was introduced into Europe around 1570 and eventually it became a staple food in Northern Europe by mid 1700 and most of the small scale farmers were living on a diet of potato and milk. Potato ranks fourth in the world after wheat, maize and rice (Muthoni *et al.,* 2009). In Kenya, potato is used for fresh consumption and as potato chips, crisps and frozen processed products. Dutch Robijn variety is popular for processing into crisps while Tigoni variety is used for frozen chips and mashed food (Randall *et al.,* 2010).

Potato seeds can be purchased from Research Centers such as the Kenya Agricultural and Livestock Research Organization (KALRO) and seed farmers working with the Ministry of Agriculture on seed positive selection (Kabira *et al.,* 2006). Potato tubers (seeds) are multiplied from mini-tubers produced using aeroponics, aquaponics, clonal multiplication and other tissue culture techniques (Mbiyu *et al.,* 2012). The seed tubers are then multiplied in the field for three generations with routine indexing for viruses during pre-basic seed production (Muthoni *et al.,* 2010). The seed sector is not able to supply enough seed to potato farmers in Kenya (Wageningen UR, 2013) and therefore, the country imports seed potato of many varieties from Holland and, these include Toluca, Lady Amarrila, Carorus, and Lady Rosseta among others. The varieties are tested at Kenya Plant Health Inspectorate Service (KEPHIS) laboratories for bacterial, viral and nematode diseases followed by National performance trials (KEPHIS, 2011).

Although potato is among the most important crops in Kenya, the country has been experiencing a shortage in supply of quality seed potato (Schulte-Geldermann *et al.,* 2012). Due to this problem, the need to import potato seed was realized leading to direct import of seed potato from Netherlands to solve the constraint of potato production. During import, phytosanitary issues are put into consideration and it has become necessary to evaluate introduced varieties for adaptability to the local production conditions including late blight (KEPHIS, 2011).

Late blight is an important disease of potato in the world. The causal organism of late blight (*Phytophthora infestans*) produces many sporangia that cause heavy damage to the crop through foliage infection. The pathogen is spread by wind and through infected seed. The fungus can survive in infected potato tubers becoming a seed borne pathogen (Duggar, 2002). It can survive as oospores in the soil in the infected site and the pathogen becomes soil borne. Race A2 mating type of *P. infestans* is found in Europe and North America and produces oospores that can survive in the soil for many years and germinate to initiate early epidemics. Sexual reproduction increases recombination within the population leading to production of oospores which remain viable in the soil or infect tubers making them a source of primary inocula. In Kenya, race A1 of *P. infestans* causing late blight is present (Duggar, 2002) and the disease is managed through cultural control methods by most farmers namely, early planting, ridging of crop, weeding, certified potato seeds and fertilizer use. Use of fungicides is limited in small scale potato production due to high costs of inputs (Janssens *et al*., 2014).

1.2 Problem statement

Potato is the second most important food crop in Kenya after maize (Felix *et al*., 2010, Were *et al.,* 2013). The crop is a good source of carbohydrates, vitamin C, potassium and an excellent source of fibre. There is an increase in demand for potato resulting from increase in population growth and diversification of

eating habits and this has led to increased potato production by farmers in Kenya. As a result, farmers have started growing potato in areas that traditionally were not used for potato cultivation. These areas include Bomet, Bungoma, Elgeyo Marakwet, Kiambu, Meru, Narok, Nyandarwa, Nyeri, Taita-Taveta, Trans Nzoia, Uasin Gishu and West Pokot Counties (Onditi *et al.,* 2012). The major constraints to potato production include high incidence of pests and diseases and lack of sufficient quantities of certified seed tubers (Schulte-Geldermann *et al.,* 2012). Currently, there is a bilateral cooperation between the Kenyan and Netherlands governments to improve the potato seed sector. This has led to importation of potato seed. However, the health status of these imported materials needs to be routinely verified. There is a knowledge gap on the reaction of the imported tubers to diseases such as late blight under Kenyan conditions. This research seeks to fill the information gap that exists on health status of the imported seed potato germplasm and reactions of the potato varieties to late blight under Kenyan conditions.

1.3 Justification

Kenya has experienced problem in potato production due to the lack of quality seeds and only 1 to 2% of potato cultivated area is planted with certified seed potato. In the last 5 years more farmers have entered into potato business increasing production from 2.3 to 2.9 metric tons and this increased demand for certified seed (Janssens *et al.,* 2014). Local seed multiplication systems have not been able to produce sufficient quantities of basic seed for further multiplication and therefore, the seed sector has not been able to develop into an effective seed supply system to serve a wide range of potato farmers (Wageningen UR, 2013). It is on this basis that the Ministry of Agriculture requested the Netherlands Government to cooperate in development of the potato seed sector in order to increase the output of certified seed. The cooperation is encouraged to improve on food security and agribusiness development intended to contribute to higher income for participating small and medium potato farmers in Kenya (MOA, 2005). The cooperation has led to importation of seed potato however, it is required by law to

import disease free tubers and also to evaluate suitability of introduced varieties for performance under local production conditions including evaluation of reaction to diseases such as late blight. This evaluation will bridge the existing knowledge gap on the reaction of the imported potato seed to late blight. The information generated from this study will be used to advise potato growers on the reaction of potato varieties to late blight with a view of managing the disease in potato for increased production.

1.4 Main objective

To increase potato production by ensuring freedom from diseases of imported germplasm and identification of resistance to late blight disease.

1.4.1 Specific objectives:

1. To assess seed potato production systems and disease prevalence in selected potato growing regions in Kenya
2. To determine the levels of disease infection of imported and locally bred potato seed
3. To evaluate imported potato germplasm for reaction to late blight disease under greenhouse and field conditions in Kenya

1.5 Research hypotheses

1. Major potato growing regions in Kenya are contaminated with diseases and disease prevalence is high.
2. Potato seed used by farmers is infected with viruses, bacteria and fungi namely *P. infestans*
3. Imported potato germplasm is susceptible to late blight under Kenya conditions

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Origin and botany of potato

The origin of potato (*Solanum tuberosum* L.) is in the Andean highlands of South America (Razdan, *et al.,* 2005, Randall, 2010). The crop is in the family *Solanaceae* which includes tomato, pepper, egg-plant, tobacco, and weeds like nightshade. The crop is propagated vegetatively from tubers ("seed"). Growth development of the crop is in five distinct life stages namely sprout development, vegetative growth, tuber initiation, tuber bulking and maturation. Sprouts develop from eyes on seed tubers, leaves and branches and stems grow from above ground nodes along emerging sprouts. Roots and underground stems (stolons) develop at below ground nodes. During early growth, plant obtains energy from seed tubers. Tubers form at stolon tips which are controlled by growth regulating hormones produced by the plant. Tuber initiation takes 10-14 days in most cultivars. During tuber bulking, tuber cells expand with accumulation of water, nutrients and carbohydrate, if growth factors are not limiting (Randall *et al.,* 2010).

Pollination in potato crop is by insects that require pollen because the plants do not produce nectar. Insects attracted by purple, yellow and blue flowers vibrate potato plants to release pollen. Pollination is very important in breeding of potato to produce true potato seeds and it is done by hands. In potato production, tubers are used (Kabira *et al.,* 2006).

2.2 Economic importance

Potato ranks fourth in the world food production, after wheat, maize and rice. It is an important crop in Kenya and the whole world and it is used for consumption and commercial purpose. Tigoni variety is used for making french fries, frozen chips, stew and mashed food (Muthoni *et*

al.,2014). Dutch Robjin variety is popular for processing crisps (Randall *et al.*, 2010). Currently, many varieties of potato are grown in Kenya and these includes Asante, Tigoni, Kenya Mpya, Sherekea, Nyayo and many others. The seed potato can be purchased from Research Centers such as KALRO and seed farmers working with the Ministry of Agriculture (Kabira *et al.*, 2006).

In Kenya potato is grown by 800,000 farmers and most of them are small-scale farmers. Small and medium-scale farmers have more than 80% of potato acreage under potato production. Nearly all Kenyan potatoes are consumed locally and consumption is estimated by total production. Eight hundred thousand small scale farmers are growing potato in Kenya and this implies that 800,000 households depend on potato for food. Potato sub-sector contributes fifty billion shillings to Kenya economy (Ksh 50 billion) (Janssens *et al*., 2014).

2.3 Ecological requirements

Potato does well in highlands at 1500-3000m above sea level with rainfall of more than 600 mm. The soils should be deep, well drained and fertile with a pH of 5.5 -6.0. Daily temperature of 15-18^{0}C is ideal. Temperature above 21^{0}C has adverse effect on growth of potato. The sites should not be hilly to make hilling easier (Kabira *et. al.*, 2006).

2.4 Cultivation

Potato is cultivated in Kenya as a food crop for local consumption as potato chips, crisps, French fries and frozen processed products. The crop is also used in stews or mashed with maize and beans or other pulses and green vegetables are added (Muthoni *et al*., 2014). This is an important crop and ranks fourth in the world food production, after wheat, maize and rice. The "seeds' for planting are normally the size of chicken eggs with a diameter of 35-45mm. Pre-sprouted seeds

for early emergence and uniform growth are necessary (Kabira *et al.*, 2006). This reduces late season diseases and insect pests. Depending on varieties, sprouts of 8-10 weeks after harvest are preferred. The spacing of potato is 30x75cm and this allows scouting for diseases and insect pests. DAP-18:46 at 500kg /ha is applied in the furrows and thoroughly mixed with soil before planting. Single super phosphate is applied at 500kg/ha and then crops are top-dressed with calcium ammonium nitrate or NPK fertilizers during ridging as a source of nitrogen at 300kg/ha (Randall *et al*., 2010). Well decomposed manure is used at 5-10 tons /ha. Weeding begins 6 weeks after planting and 2 weeks after germination hilling is done. Spraying against pests using fungicides and insecticides is recommended. The crop matures in 3-4 months and can yield up to 40 tons per ha (Kabira *et al*., 2006, Denis *et al*., 2010, Randall *et al.*, 2010).

2.5 Constraints to potato production

There are many constraints to potato production in Kenya namely, low soil fertility, lack of certified seed, low market price, disease and insect pests (Janssens *et al.*, 2014). The crop is attacked by many diseases and pests which include weeds, insects, nematodes, bacteria, viruses, viroids and fungi. Late blight caused by *P. infestans*, is an important disease of potato (Duggar, 2002). The yield losses associated with late blight is 40-50% in Kenya (Njuguna *et al*., 1998).

The second group of diseases that affect potato consists of viruses. All viruses infect developing tubers and are carried over from season to season in "seed" tubers. Viruses of potato crop include potato mosaic virus, potato leaf roll virus, potato virus Y, potato virus A, potato virus S, potato virus M and potato virus X. Beetles, aphids, and leaf hoppers transmit viral diseases. Viruses and viroids can also be spread mechanically. Viruses like PVX can remain infective in the soil and

machinery for several hours (Kabira *et al.,* 2006, Gallenberg *et al*., 2007). Potato health can also be limited by environmental factors and competition from weeds (Randall *et al.,* 2010).

Research indicated that several potato varieties were tested for resistance to late blight, viruses and yields and released to farmers (Onditi *et al.,* 2012). Tigoni and Asante were released in 1998 and Kenya Sifa, Kenya Karibu, Kenya Faulu and Kenya Mavuno in 2002 (Lung'aho *et al.,* 2006). In 2012, Sherekea, Kenya Mpya and Purple Gold were released (Onditi *et al.,* 2012). Sherekea was resistant to PVY and PLRV, Kenya Mpya to PVY and PVX and both varieties had high resistance to late blight. Yields were high Sherekea yielding 45 to 50 t/ha and Kenya Mpya 40 to 45t/ha (Onditi *et al.,* 2012). The following are some of the important diseases that affect potato health.

2.6.1 Late blight

Late blight caused by *P. infestans* is an important disease of potato and infection is by both sporangia and zoospores (Alexopoulos *et al*., 1988; Drenth *et a*l., 2001; CABI, 2016). The disease was not known in Europe until it devastated the potato crop in Ireland in 1945 and 1946. This caused famine resulting to death of a million people and 1.5 million migrated to North America (Randall *et al*., 2010).

Late blight is among the most important diseases of potato and most varieties are susceptible to the disease. The disease spreads rapidly under cool humid weather through infected tubers and causes great economic losses particularly during the long rain seasons. The disease attacks the leaves, stems and in the advanced stages of infection may enter tubers. Symptoms include water soaked lesions on the foliage, which turn brown when dry and black when wet. The spots can also occur on the tips of the stems, which turn black and die (Duggar, 2002). On the underside of

the leaf, the fungus produces a white moldy growth seen more clearly at the edges of the spots. An infected crop loses all foliage in a few days while infected tubers have sunken, grey spots, slightly depressed brown-purplish skin that reveal a reddish-brown rot below the surface when cut (Schumann *et al.*, 2005). This injury is frequently followed by secondary infection of the fungus. In Kenya, the yield losses associated with late blight is 40-50% (Njuguna *et al*., 1998).

2.6.2 Bacterial wilt

Bacterial wilt **(***Ralstonia solanacearum***)** is also known as brown rot and affects more than 30 plant species, the most susceptible being potato, tomato, eggplant, pepper and groundnut. Limited availability of high-quality seed and lack of farmer knowledge on proper agronomic practices for its control is a major factor in its spread (Kabira *et al.*, 2006). BW is spread by infected seed tubers, crop residues, contaminated surface water used for irrigation, contaminated soil adhering on shoes, and tools (including tractors) (Zachman., 2000; Muthoni *et al.*, 2014). The disease enters potato roots through wounds made by tools during post-emergence cultivation and through attack by nematodes and insects (Zachman., 2000; Randall *et al*., 2010). Infected plants wilt even when the soil has sufficient moisture. The leaves droop and eventually the plants die. To ensure that the damage is not due to insects, the tuber from the wilted plant is cut and squeezed and if a white mass of bacteria oozes out, BW is likely to be the cause of wilting (Zachman., 2000). Latent infections, however, cannot be detected by this test but, requires to be detected through molecular techniques (Pastrik *et al.*, 2000; Priou., 2001). BW disease has no effective means of control, no known chemical control measure and the pathogen has a wide range of hosts. The disease persists for long in the soil and affects over 70% of potato farms causing yields loss of between 50 to 100%. BW can cause total loss of a crop and prevent the use of land for potato production for several years (Muthoni *et al.,* 2012).

2.6.3 Viruses

Viral diseases are also important in potato production. They are associated with potato planting stock. They include potato mosaic disease, potato leaf roll, potato virus Y, potato virus X, potato virus A, potato virus S and potato virus M. Insects such as beetles, aphids, and leaf hoppers are reported to transmit these viral diseases (Randall *et al.,* 2010).

Viral diseases cause degeneration of seed potato with leaf roll and several mosaic viruses are the main causes of seed degeneration. Areas meant for seed production must be carefully selected to minimize infection. Potato leaf roll virus (PLRV) is spread by aphids, mainly the green peach aphid (*Myzus persicae*) (Mowry, 2005). The virus is also spread through infected tubers, potato aphids and diseased volunteer plants. Symptoms of PLRV appear 2-3 weeks after crop emergence (Randall *et al.,* 2010). In primary infections, symptoms first appear at the top of the plant where the leaves roll inwards and turn pale yellow and some may develop yellow margins. In secondary infections, the entire plant is affected; all the leaves roll inward, especially at the base of the plant than at the top where the old leaves are clearly rolled (Mary *et al.,* 2005). Growth is stunted and plants develop small tubers. When these tubers are used as seeds after maturity, the plants are stunted and the crop produces very low yields.

Potato virus Y (PVY) is among the mosaic viruses and it is transmitted by aphids and is also spread through infected tubers. It is easily transmitted and can cause major yield losses alone or in combination with other viruses such as PVA, PVX or PVS (Mary *et al.,* 2005). Mild mosaic caused by potato virus A (PVA) is common in all potato producing areas. High yield losses may occur in some varieties when PVA occurs in combination with PVY or PVX. Potato virus A is similar to PVY in many aspects and it is generally less severe in certain cultivars (Randall *et al.,* 2010). It causes mosaic (sometimes severe) and crinkling, and leaves may appear shiny. Potato

virus X is transmitted through infected tubers and by contact (not by aphids) and causes mosaic. Infection may be mild in certain cultivars and it is frequently latent (Randall *et al.,* 2010).

Potato virus S is common and may cause mild symptoms. It has little effect on yield and it is transmitted through infected tubers, by contact and by aphids. Infection is usually latent, although some cultivars react with mild mosaic. Potato virus M is less common than PVY, PVX or PVS, and little is known on its effects on yield. It is spread by infected tubers and it is transmitted by contact and by aphids. The virus is latent in some cultivars although in others it causes a mild mosaic or severe mosaic and leaf crinkling (Mary *et al.,* 2005). Aphids cause direct and indirect damage to the potato plants by colonizing the underside of leaves, sucking plant sap that weakens them, interfere with photosynthesis due to fungal growth and sugar secretion produced by aphids. The aphids transmit viruses to health plants such as PVX (mottling), PVS and Potato Mosaic viruses that include PVY, PVA and PVM. Monitoring as warning system is important (Kabira *et al.,* 2006). In Kenya, viral infection causes yield loss from 60.6 to 80.1% during long rains and 64.5 to 78.0% during short rains seasons (Lunga'ho *et al.,* 2007).

2.6.4 Other diseases

Potato is also affected by other diseases such as early blight (*Alternaria solani*), black scurf (*Rhizoctonia solani*), black leg caused by two closely related bacteria *Erwinia carotovora* subsp. *carotovora* and *Erwinia carotovora* subsp. *atroseptica* but these are of minor importance in potato production in the tropical countries. These two subsp. cause black leg, aerial stem rot and tuber soft rot of potato. *Erwinia carotovora* subsp. *atroseptica* causes blackleg and it is associated with potato growing in temperate climates. *Erwinia carotovora* subsp. *carotovora* has a wide host range that include most vegetables (Denis and Mary, 2010).

2.7 Management of potato diseases

2.7.1 Late blight management

2.7.1.1 Cultural practices

Certified potato tubers have been used for planting to avoid initial inoculum. Planting early is encouraged so that the crop matures before late blight sets in. Use of fertilizers is put into consideration in disease management strategies. Disease development depends on nutrition of the plant. High dosage of nitrogen leads to higher susceptibility of the plant. Potassium influences the production of sporangia and therefore fertilizer use in potato production should be low especially nitrogen fertilizers like NPK used in top dressing (Mackerron *et al.,* 2000). Hilling or ridging the crop reduces the inocula by covering fallen leaves carrying *P. infestans* that may continue attacking the crop. Weeding, destruction of potato remains after harvesting and planting trees as a barrier on one side of the field to prevent spores spread by wind help to reduce inocula. Removal of volunteer potato plants during crop rotation before planting potato is equally important (Kabira *et al*., 2006). Monitoring programmes should be put in place to help in early detection of the disease. Scouting of potato fields for late blight should start before planting to check presence of sources of inocula and continue weekly until the harvest time. Varieties developed with some resistance are used in disease management (Agrios, 2004).

2.7.1.2 Chemical control measures

Late blight disease is controlled using fungicides and spraying when the plants are 10 cm tall. Spraying is carried out as a preventive measure, especially if the variety grown is susceptible (Kabira *et al.,* 2006). Spraying commences when the first disease symptoms are noticed. It is important to spray on both sides of the leaves. Use of clean seed and application of fungicides

such as Mancozeb (Dithane M45) and Metalaxyl-M (Ridomil) to all parts of the plant reduces disease incidence (Hooker *et al.,* 1981; Kabira *et al.,* 2006; Muthoni *et al.,* 2013).

2.7.2 Bacterial wilt management

Bacterial wilt can only be controlled using an integrated disease management approach involving the planting of healthy seed in clean soil, planting tolerant varieties, rotation with non-host crops and in fields previously not cropped with tomato, egg-plant and pepper. Non-susceptible crops include cucumber, beans, lucerne, barley, maize, sorghum, wheat, onion, beet, turnip, radish, carrot, parsley, sweet potato, strawberry and grasses (Denis *et al.,* 2010; Zachman., 2000).

2.7.3 Management of potato viral diseases

Potato leaf roll virus (PLRV) can be avoided by planting healthy plants and eliminating diseased plants through roguing while mosaic viruses can be avoided by selection of clones during seed multiplication. Roguing is useful only when obvious symptoms develop and most potato viruses are transmitted by aphids (*Aphididae*) which belong to *hemimetabolous* insects order *Hemiptera* Suborder *Homoptera* (*Sternorrhyacha*) (Mayer, 2016). Systemic insecticides decrease spread by aphids within the crop but do not prevent infection by aphids from other fields. Direct control of potato viruses is not practical but spraying with systemic insecticides can control aphid. Spraying and roguing controls the viruses in seed crop. Only high-grade stock should be planted when producing seed (Kabira *et al.,* 2006).

Seeds should be planted in fields without volunteer potatoes which could harbour aphids and potato viruses. Diseased plants and haulms should be removed and destroyed early. Only insecticides that kill aphids immediately after emergence should be applied (Kabira *et al.,* 2006).

CHAPTER THREE

3.0 Potato seed production systems and disease prevalence in major potato growing regions in Kenya

3.1 Abstract

Potato is an important crop in Kenya for domestic consumption and as cash crop. Farmers obtain seed from various potato seed systems which vary between different areas. The objective of this study was to elucidate potato seed production systems and farming practices and evaluate the prevalence of bacterial wilt and potato leaf roll virus. A survey was carried out between April and May 2014 in five major potato growing counties in various agro-ecological zones (AEZs); Meru (UH3 & LH4), Nyandarua (UH3), Molo Elburgon (UH2), Nyeri (LH4 & LH3) and Kiambu (UH3). An open ended questionnaire was administered to fifty nine (59) farmers to collect information on area under potato, potato production systems, farmers' practices and prevalence of bacterial wilt and potato leaf roll. Other information that was captured included the global positioning of the farm (latitude, longitude and altitude), farm size, crops grown and crop cycle. The area of land allocated to potato production per farm varied from an average of 0.3 ha to 0.7 ha, with Nakuru having the highest area of land allocated to potato production of 0.7 ha, Kiambu 0.5 ha, Meru 0.4 ha, Nyandarua and Nyeri with a low acreage of 0.3 ha. About 80% of farmers in Kiambu county obtain potato seed from KALRO Tigoni, while most of the farmers in the other four counties, use their own farm saved seeds, buy from the local market and from their neighbors. A few (10.3%) farmers from Meru obtain potato seed from Kisima Farm, while those in Nakuru (20%) source it from Turi Farm and in Nyandrua, 15% source it from KARLO Njabi-ini. Some farmers avoid replanting the same seed (renew) and obtain new potato seed from certified source with Kiambu leading with about 80% followed by Nakuru (27.3%), Nyandarua (18.2%) and Meru (13.6%) respectively. Bacterial wilt disease was detected in all counties with

the highest prevalence in Nyeri at 90.0%, followed by Meru and Nyandarua each with 81.8%, Nakuru 63.6% and Kiambu 60.0%. Potato leaf roll virus was also detected in all the five counties, the highest prevalence of 90.9% recorded in Nyandarua and 36.4% in Nakuru County. The study revealed that farm sizes under potato production are small and farmers are not able to practice long quality crop rotation. Majority of them do not plant certified seed and therefore bacterial wilt and viruses are prevalent and widespread in all the five counties.

3.2 Introduction

Potato is an important crop in Kenya and it ranks fourth in food production among the four major crops grown that include rice, wheat and maize (Hoffler, H., 2008, FAO, 2008). It provides high incomes for small scale farmers and it is a potential source of employment to many people both directly and indirectly (Mbaka *et al*., 2013) Seed quality is affected by biological aspects such as the level of disease infection and physiological age of seed tuber. Tuber seeds always harvested from the same farm and planted continuously for several years show degeneration which is a result of viruses and virus-like organisms which accumulate in tubers. The major viruses infecting potato and lowering potato seed quality are mainly potato leaf roll virus, potato virus Y, potato virus X, potato virus A and potato virus M (FAO, 2008). Bacterial wilt is a serious threat to potato production due to limited strategies to control the disease effectively and farmers are abandoning cultivation of the crop due to the disease (Mbaka *et al.*, 2013).

Commercial quality requires that seed tuber size be 40- 50gm to guarantee quality, seed should be free from bacteria, viruses and fungi which may lead to seed degeneration (FAO, 2010). In case of certified potato seed, production starts with *in-vitro* plantlets from meristematic tissue in a tissue culture laboratory. Plantlets are multiplied and after hardening, they are planted in sand media in greenhouses to produce mini-tuber seeds which are multiplied in the field for three generations to produce basic seed. The seed is planted for one generation at Tigoni and other substations of KALRO. KALRO contracts farmers who multiply these certified seed for sale to other farmers growing potato ((Mbiyu *et al.*, 2012; Muthoni *et al.*, 2013).

In seed multiplication system, the contracted farmers produce potato tuber seeds from clean starter seed provided by KALRO. The clean starter seeds undergo inspection by authorized regulatory organization (KEPHIS) before they are released for multiplication to be sold to potato

growers (FAO, 2010). In positive selection, seed system (quality control seed system), potato plants in the field that are free from diseases are selected and earmarked as mother plants and used for seed multiplication. These are then used as seed potato for planting by farmers in the subsequent season. This innovation was developed to support farmers improve the quality of farmers' seed potato (Gildemacher *et al.*, 2011). There has been lack of enough certified seeds to be supplied to farmers and this has forced farmers to plant seeds from their own farms. These poor quality seeds increase the rate of spread of seed-borne diseases such as bacteria wilt leading to high incidence and prevalence. Previous research indicates that bacterial wilt is found in all the potato growing areas of Kenya affecting 77% of potato farms followed by late blight (69%) (Kaguongo *et al.,* 2010; Peeters *et al*., 2013).

Though rotation can reduce disease incidence, many farmers are not able to observe the recommended period (3 years) of rotation due to the small sizes of the farms (Muthoni *et al.,* 2013). Cropping cycle using seeds from the same farms without renewing from certified seed sources also contribute to the accumulation of seed-borne diseases namely bacterial wilt caused by *R. solanacearum* and viral diseases such as PVY and PLRV which are the major potato diseases. Poor quality seeds reduce potato quality and yields (Gidemacher *et al.,* 2009; Tahat *et al*., 2010).

3.3 Materials and methods

A survey was conducted in the major potato growing counties in Kenya namely Nyeri, Nyandarua, Meru, Nakuru and Kiambu between April and May 2014 (Figure 3.1). The farms surveyed ranged in altitude from 2079 to 2617 meters above sea level (masl). The agro-ecological zones surveyed for the various counties were Meru-UH3 (wheat and barley zone), Nyandarua –UH3 (wheat-barley zone), Molo Elburgon – UH2 (pyrethrum – wheat zone), Nyeri-

LH4 & LH3 (cattle-sheep zone) and Kiambu-LH3 (cattle-sheep zone) (Jaetzold *et al.,* 2007). In these counties potato crop is grown for food consumption and commercial purposes. In each of the five surveyed counties, farms were selected at random in the counties about 2-4km away from each other.

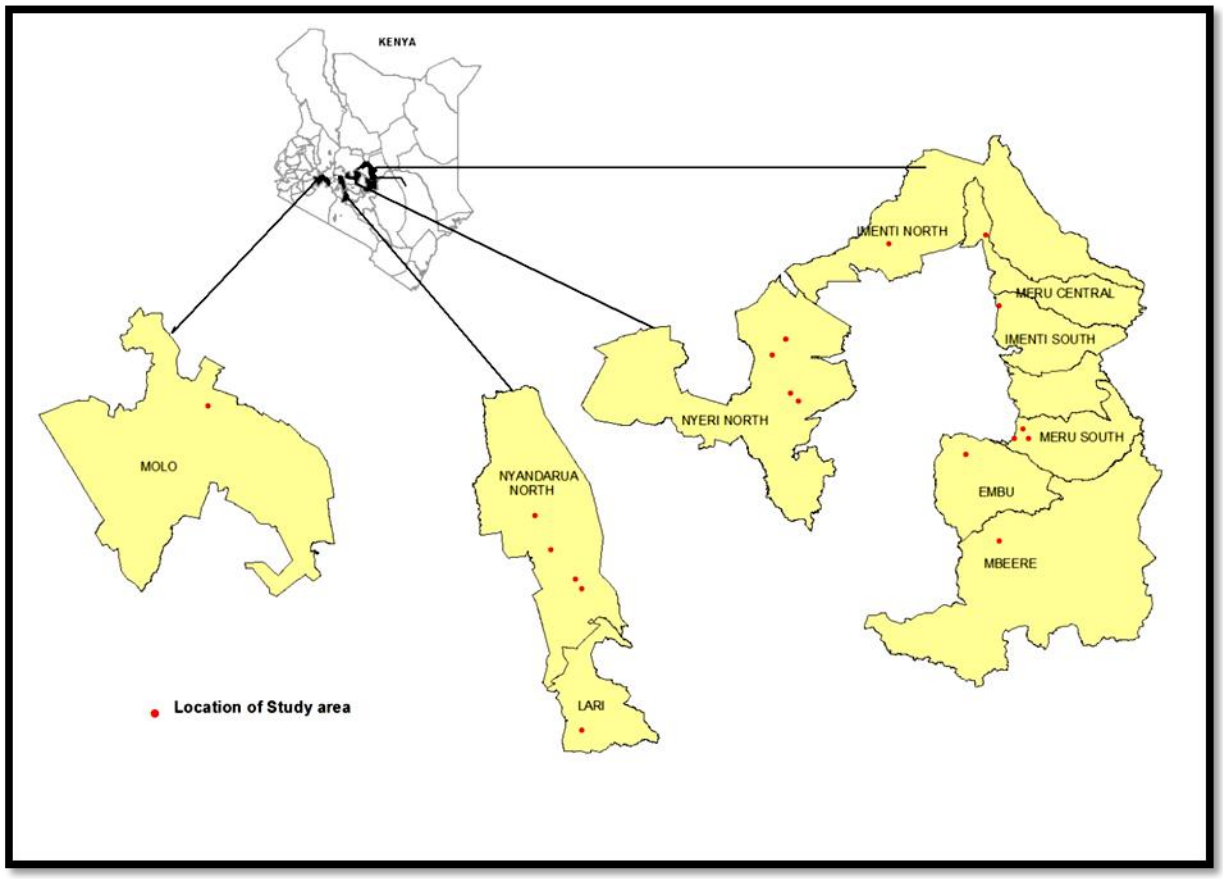


Figure 3.1: Potato production areas in Kenya

During the survey, bacterial wilt and potato leaf roll virus disease incidences were recorded per sampled farm. Meru county had the highest number of farms (22) because the county is a major potato producing county in the Mount Kenya region (Ng'ang'a *et al.*, 2003; Muthoni *et al*., 2013). During the survey, a total of 59 farmers were interviewed as follows Nakuru (11),

Nyandarwa (11), Meru (22), Nyeri (10) and Kiambu (5). Information was collected using an open ended questionnaire to allow individual farmers to express themselves and to be able to gather more responses on the potato seed production systems (Farrell., 2016). The information collected included farmers' practices such as source of seeds, seed renewal, cropping cycle, area under potato production and disease incidence and prevalence. The collected data were recorded and analyzed using Statistical Package for Social Sciences (SPSS Inc. (2009). Visual assessment of the fields showing symptoms of bacterial wilt (Tahat *et al*., 2010) and potato leaf roll virus was done. Disease severity and prevalence per sampled farm were recorded. Number of farms with disease were divided by total number of farms surveyed and multiplied by one hundred to get disease prevalence (Kinyua, *et al.,* 2012).

Information on geographical positioning system (GPS) was collected namely elevation, longitude and altitude (Peter *et al.,* 2015; U.S. Government, 2016). This information was to facilitate mapping the position of the sites of survey using geographical information system (GIS) (U.S. Government, 2016). Samples were collected from farmers, certified seed and registered seed merchants and taken to KEPHIS laboratories at Muguga and analyzed for latent infection of bacterial wilt and viruses (Saddler et al., 2005; Mwangi *et al*., 2008; Tahat *et al*., 2010; Genin *et al*., 2012).

3. 4 Results

3.4.1 Potato growing in the various counties

A total of fifty nine (59) farmers were interviewed and these comprised of 37.3% from Meru, Nakuru 18.6%, Nyandarua 18.6%, Nyeri 16.9% and Kiambu 8.5% (Table 3.1).

3.4.2 Acreage allocated to potato crop

The land allocated to potato production varied from 0.3 ha to 0.7 ha, with Nakuru having the highest acreage under potato production of 0.7 ha followed by Kiambu with 0.5 ha, Meru 0.4 ha, Nyandarua 0.3 ha and Nyeri-Kieni East with the lowest acreage of 0.3 ha (Table 3.1).

Table 3.1: Average farm size (ha) under potato production in the five counties

County	Mean farm size(ha)	Standard Error of mean
Nakuru	0.7	0.02
Kiambu	0.5	0.12
Meru	0.4	0.06
Nyandarua	0.3	0.06
Nyeri	0.3	0.06
Overall mean	**0.4**	**0.03**

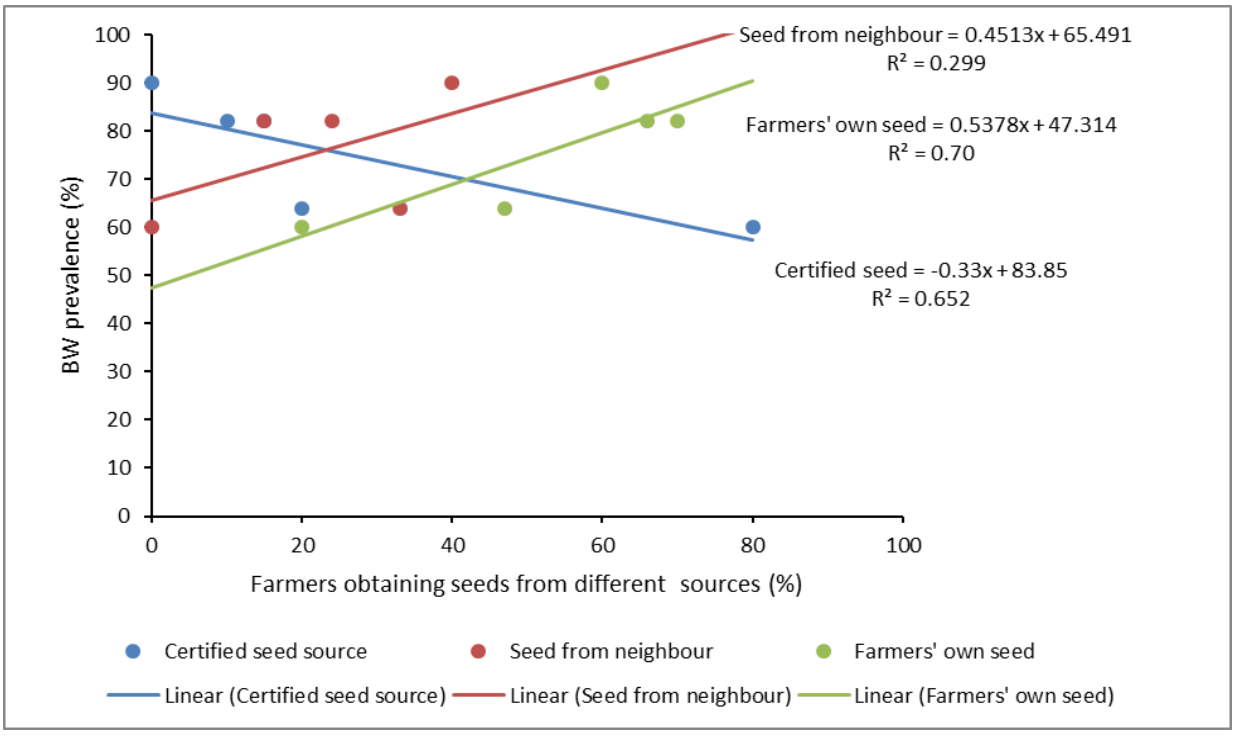
3.4.3 Source of potato seed planted by farmers

Eighty percent (80%) of farmers in Kiambu County obtain seed potato from KALRO Tigoni, a few (10.3%) farmers from Meru obtain potato seed from Kisima Farm, while in Nakuru 20% of farmers source seed from Turi Farm and Nyandrua 15% of the farmers' source seed from KALRO Njabi-ini (Table: 3.2). Farmers from the five counties also obtain a high percentage of seeds from their own farms with Nyandarua leading with 69.2%, followed by Meru 65.5%, Nyeri 60%, Nakuru 46.7% and Kiambu 20%. Farmers also buy seeds from neighbours and markets with Nyeri leading with 40%, Nakuru 33.3%, Meru 24.1% and Nyandarua 15.4% (Table: 3.2). There was a correlation between seed source and disease incidence with Kiambu and Nakuru having low bacterial wilt and potato leaf roll virus compared to the other counties. The more the use of certified seed the less the BW prevalence and R^2 value was 0.6529 while PLRV

prevalence increased with less use of certified seeds and R^2 value was 0.4251. Regression analysis showed a relationship between increase in certified seed use with decrease in disease prevalence. Increase in the use of on farm and neighbour seed sources showed an increase in disease prevalence (Figure 3.2 & 3.3).

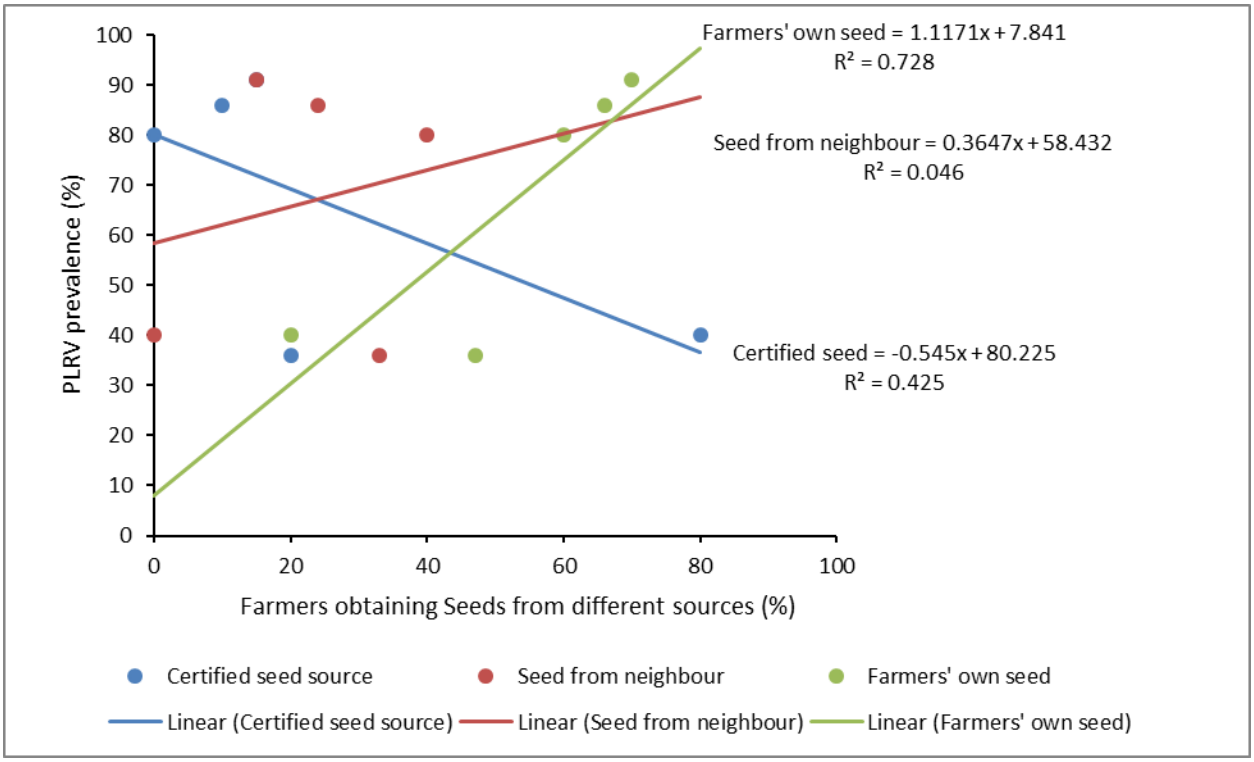
Table 3.2: Proportion (%) of farmers obtaining seeds from different sources in the five counties

Seed Source	Kiambu	Meru	Nakuru	Nyandarua	Nyeri
Certified seeds	79.0	10.0	20.0	15.0	1.0
Neighbour/market	1.0	24.0	33.0	15.0	39.0
Own farm	20.0	66.0	47.0	70.0	60.0



Key: BW Bacterial wilt

Figure 3.2: Correlation between bacterial wilt prevalence and number of farmers obtaining potato seeds from different sources.



Key: PLRV Potato leaf roll virus

Figure 3.3: Correlation between potato leaf roll virus prevalence and number of farmers obtaining potato seeds from different sources.

3.4.4 Seed Renewal

Some of the farmers interviewed renewed potato seed from certified seed source after two years with Kiambu leading with 80% followed by Nakuru 27.3%, Nyandarua 18.2% and Meru 13.6% of respondents. In Nyeri Kieni -East 1.0 % of farmers renew seeds from reliable sources (Table: 3.3 & Figure 3.4). The study showed that 60% and above of the farmers interviewed did not renew the potato seed from certified seed sources but used seeds from their own farms or purchased them from their neighbours. Use of uncertified planting seed increases seed borne diseases like BW and PLRV. There was a negative correlation between seed renewal and disease incidence and this can be clearly seen in Nyeri County where most of the respondents did not renew seed. Regression analysis showed a relationship between increase in seed renewal with

decrease in disease prevalence (Figure 3.5 & 3.6). Nyeri had bacterial wilt incidence of 90% and PLRV of 80% which is relatively high compared to other counties like Kiambu and Nakuru with PLRV of between 36.4% and 40% respectively. Bacterial wilt disease prevalence was relatively low between 60% and 64% in comparison with Nyeri (Figure 3.6 & 3.7).

Table 3.3: Proportion of farmers who renew seed in the five counties

% Farmers	**Counties**				
	Kiambu	Meru	Nakuru	Nyandarua	Nyeri
Do not renew	20.0	86.4	72.7	81.8	99.0
Renew seed	80.0	13.6	27.3	18.2	1.0

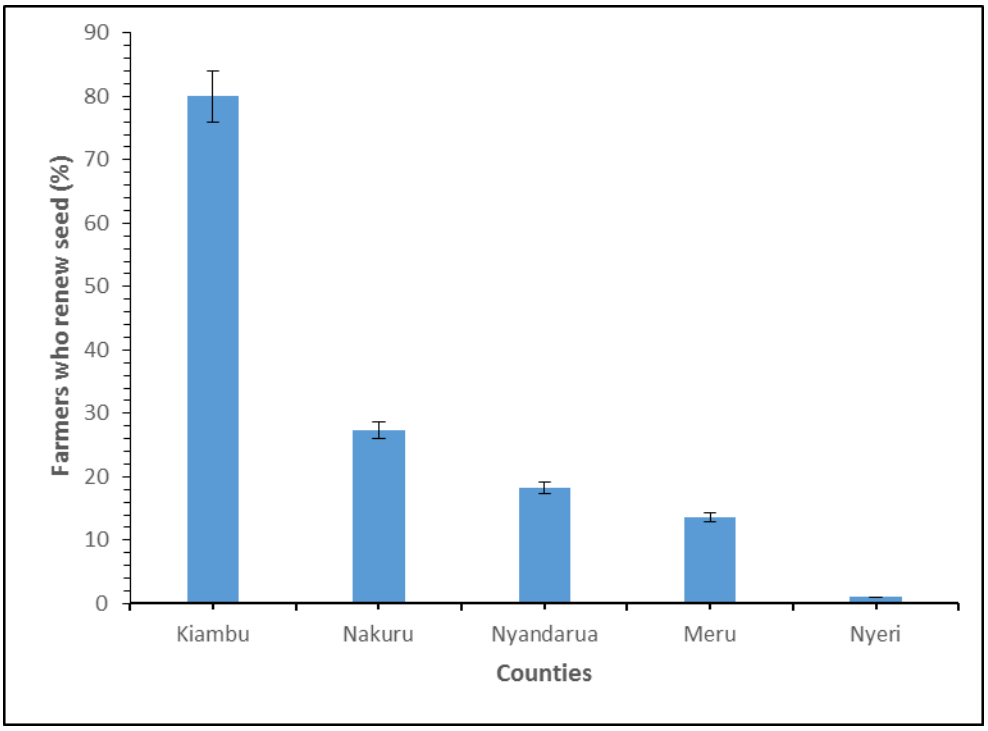
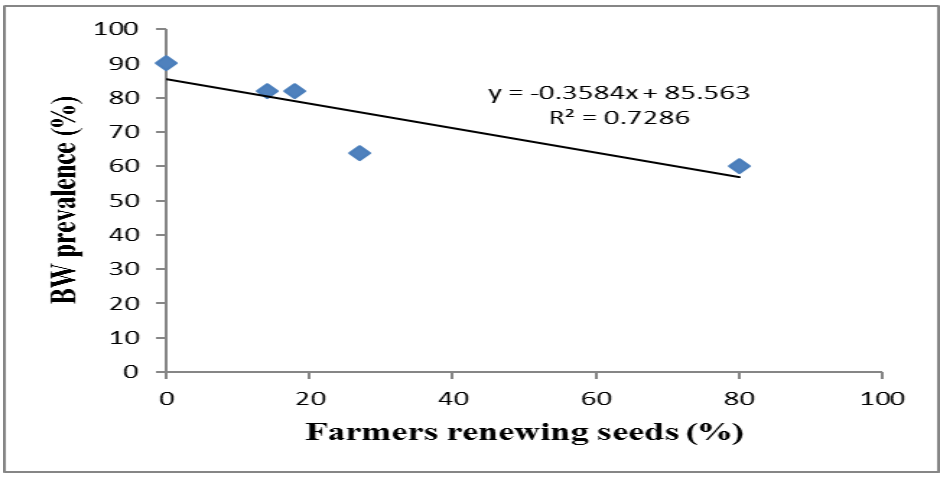
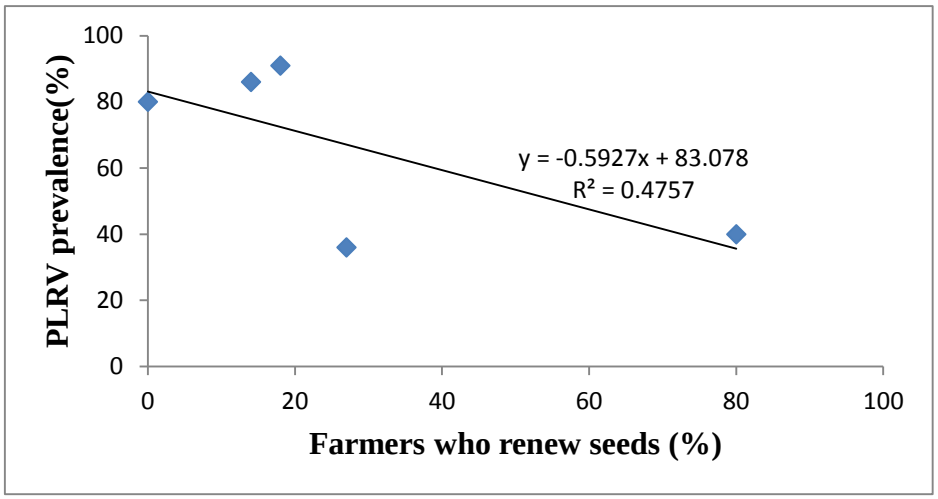


Figure 3.4: Percentage of farmers who renew seed from reliable sources in the five counties.



Key: BW bacterial wilt

Figure 3.5: Correlation between bacterial wilt prevalence and number of farmers renewing seeds.



Key: PLRV Potato leaf roll virus

Figure 3.6: Correlation between potato leaf roll virus prevalence and number of farmers who renew seeds.

3.4.5: Bacterial wilt prevalence

Bacterial wilt disease was detected in all counties and the prevalence was highest at 90% in Nyeri followed by Meru with 82%, Nyandarua 82%, Nakuru 64% and Kiambu 60.0%. There

was no significant difference in prevalence of bacterial wilt among the five counties. The study also showed that most of the farmers in Nyeri do not source seeds from reliable sources. Bacterial wilt prevalence was lower in Kiambu county at 60% than in Nyeri 90%, Meru 82%, Nyandarua 82% and Nakuru 64%. Kiambu is the county where 80% of farmers obtain seeds from reliable sources (Figure 3.7). There was a significant correlation between clean seeds use and disease prevalence where bacterial wilt disease decreased with increase in clean seed (R^2=0.6529) for BW and PLR value as R^2=0.4251.

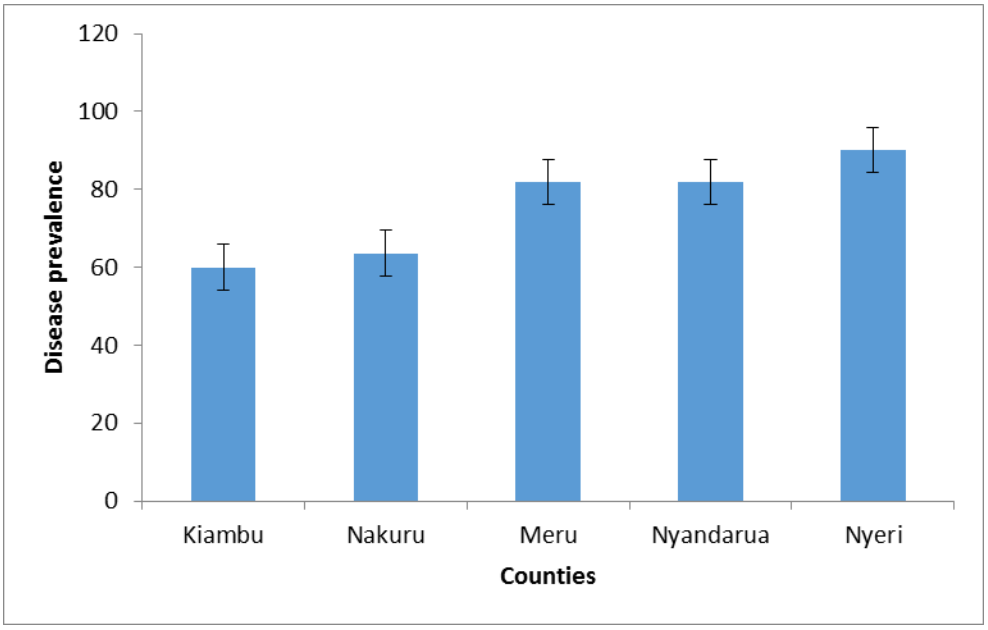


Figure 3.7: Prevalence (%) of bacterial wilt in the five counties in Kenya.

3.4.6: Prevalence of potato leaf roll virus

Potato leaf roll virus was also observed in all the five counties and Nyandarua was leading with 90.9%, followed by Meru with 86.4%, Nyeri 80%, Kiambu 40% and Nakuru 36.4% but it was low in Nakuru and Kiambu compared to all the other counties (Figure 3.8). Farmers in Kiambu and Nakuru obtain 80% and 20% of potato seeds for planting from reliable sources, respectively and also renew seeds and this may have contributed to low potato leaf roll incidence.

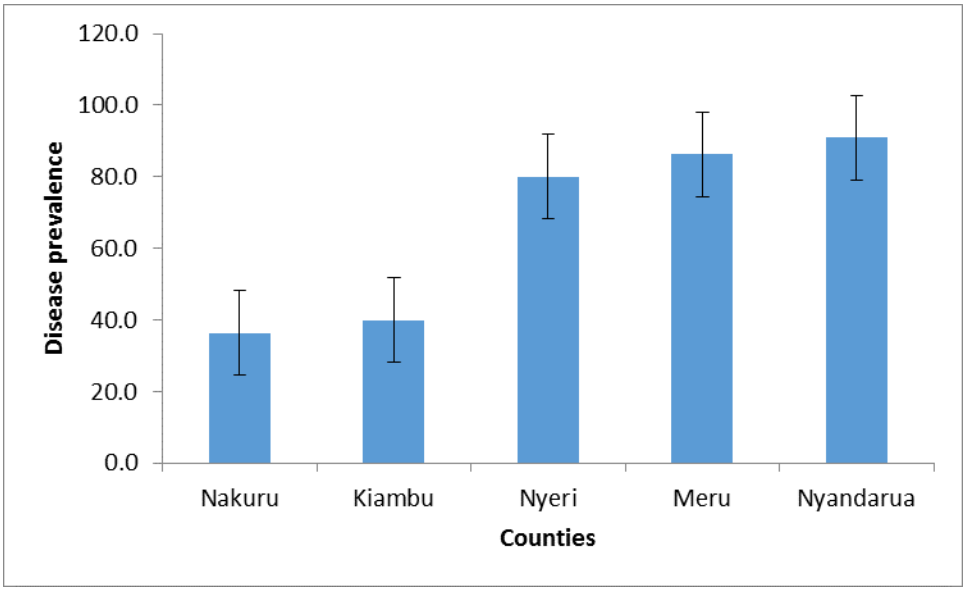


Figure 3.8: Prevalence (%) of potato leaf roll disease in five counties in Kenya.

3.4.7: Crops grown

Potato is grown in all the counties that were surveyed and Meru was leading with 56% of respondents growing the crop, followed by Kiambu with 55%, Nakuru 50%, Nyeri 48% and Nyandarua 40%. Other crops widely grown are cabbage and maize, with Kiambu having 28% of respondents growing cabbage and 15% growing maize and beans. In Meru, 12% respondents were growing cabbage and carrot and 27% maize and beans. In Nakuru county, 19% of respondents were growing maize and beans and 14% rhodes grass. Respondents in Nyandarua county also grew other crops besides potato and these are cabbages, oat and carrot while 38% of farmers in Nyeri grew maize and bean, 12% of the respondents were growing cabbages and carrot, respectively (Table 3.4).

Table 3.4: Crops grown in each of the five counties and the % of respondents growing each crop

	Kiambu	Meru	Nakuru	Nyandarua	Nyeri
Cabbage/carrot/pea	28.0	12.0	5.0	36.0	12.0
Maize+Beans/pea	5.0	10.0	19.0	10.0	28.0
Banana	10.0	17.0	0.0	0.0	10.0
Napier grass	1.0	3.0	5.0	0.0	2.0
Oat	0.0	0.0	0.0	14.0	0.0
Potato	55.0	56.0	50.0	40.0	47.0
Rhode grass	1.0	0.0	14.0	0.0	1.0
Wheat	0.0	2.0	7.0	0.0	0.0
Total	100.0	100.0	100.0	100.0	100.0

3.4.8: Cropping sequences

The most frequent crops sequences in the five counties surveyed, two year rotations (Table 3.5). In Meru county, the rotation is for two years involving potato, maize + beans or cabbage and potato. In Nyandarua, farmers also practiced two years rotation of potato, cabbage or carrot or pea and those surveyed in Nyeri did the same with potato, beans or maize, cabbage, potato. Farmers in Kiambu mainly planted potato, followed by cabbage, maize +beans and potato while at Nakuru they plant potato, maize + beans, rhodes grass and potato.

In Meru county where cropping cycle involves potato rotated with maize or cabbage, BW prevalence was 81.8% and PLRV was 86.4%. In Nyandarua BW was high up to 90.0% and PLRV was 90.9%. In Nyeri where crop rotation is between potato and maize or beans, BW incidence was 90.0% and PLRV incidence of 80.0%.

Surveyed farmers from Nyeri County did not source seed from reliable sources at all and used 60% seed from their own farms and 40% from neighbours. Kiambu and Nakuru counties had slightly lower disease prevalence compared to all the other counties. Kiambu had BW incidence of 60.0% and PLRV incidence of 40.0% and Nakuru with BW prevalence of 63 % and PLRV of 36%. In Kiambu, the rotation sequence is for two years with potato, cabbage, maize and potato while farmers in Nakuru also practiced two year rotation cycle using maize + beans or maize and rhodes grass.

Table 3.5: Cropping sequence practiced by farmers in the surveyed counties

County	Cropping cycle	Frequency	Percentage (%)
Kiambu	Potato, Cabbage, Maize, Potato	2	40.0
	Potato, Cabbage, maize & bean, Potato	2	40.0
	Potato, Maize &beans, Cabbage, Potato	1	20.0
	Total	5	100.0
Meru	Potato, Cabbage, Maize, Potato	5	22.7
	Potato, Maize, Potato	5	22.7
	Potato, Maize+ Bean, Potato	6	27.1
	Potato, Maize, Bean, Potato	2	9.1
	Potato, Cabbage, Potato	1	4.5
	Potato, Maize, Cabbage, Potato	1	4.5
	Potato, Maize, Cabbage, Wheat, Potato	1	4.5
	Potato, Wheat, Cabbage, Maize, Potato	1	4.5
	Total	22	100.0
Nakuru	Potato, Cabbage, Potato	2	18.2
	Potato, Beans +Maize, Rhode grass, Potato	2	18.2
	Potato, Beans Maize, Wheat	1	9.1
	Potato, Maize +bean, Rhode grass, Potato	1	9.1
	Potato, Maize +Beans, Potato	1	9.1
	Potato, Maize +Beans, Wheat, Rhode grass, Potato	1	9.1
	Potato, Maize Beans, Potato	1	9.1
	Potato, Rhode grass, Maize +Bean, Potato	1	9.1
	Potato, Wheat, Rhode grass, Potato	1	9.1
	Total	11	100.0
Nyandarua	Potato, carrot, Cabbage, potato	2	18.2
	Potato, Pea, Cabbage, potato	2	18.2
	Potato, Cabbage, Oat, Potato	1	9.1
	Potato, carrot, Cabbage, Oat, Potato	1	9.1
	Potato, carrot, Cabbage, Potato	1	9.1
	Potato, carrot, Oat, Cabbage, Potato	1	9.1
	Potato, Oat, Cabbage, Potato	1	9.1
	Potato, Oat, Maize, Cabbage, Potato	1	9.1
	Potato, Pea, Oat, Cabbage	1	9.1
	Total	11	100.0
Nyeri	Potato, Beans, Potato	2	20.0
	Potato, Maize, Bean, Potato	2	20.0
	Potato, bean, Maize, Cabbage ,Potato	2	20.0
	Potato, bean + Maize, Napier grass, Potato	1	10.0
	Potato, Maize +Bean, Potato	1	10.0
	Potato, Maize, Bean, Cabbage Potato	1	10.0
	Potato, Maize, Bean, Cabbage, Carrot, Potato	1	10.0
	Total	10	100.0

NB: Frequency represent the number of farmers practicing the crop cycle in the county

3.5 Discussion

The potato growing regions in Kenya fall within the altitude range from 1,200 to 3, 000 meters above sea level but worldwide, the crop is mainly grown between 1,500 and 3,000 masl. The main potato growing regions lie between mid and highland areas of Kenya including Nyandarua in Central province along the Aberdare range, Meru in Eastern province around the slopes of Mount Kenya and Molo (Lutaladio *et al.,* 1995). Potato growing regions that were surveyed lie at altitudes of between 2079 to 2617 meters above sea levels (masl) which is within the recommended potato growing regions. Research has shown that altitude contributes to disease incidence and there is reduction in bacterial wilt with increase in altitude due to the reduction in temperature (Mwaniki *et al.,* 2016; Singh *et al*., 2014). *Ralstonia solanacearum* that causes bacterial wilt is reported to survive in soil temperatures as low as 4^0C and is considered to be a warm area pathogen since growth and disease development is inhibited at temperatures below 18ºC (Champoiseau and Momol, 2009; Fajinmi and Fajinmi, 2010; Sullivan *et al.,* 2013).

During the survey, it was found that bacterial wilt was distributed in all the five counties surveyed with a higher prevalence in the high altitude areas namely Nyandarua and Meru. The prevalence of bacterial wilt was lower in Kiambu and Nakuru compared to Nyandarua and Meru which are located at a higher altitude. This was not expected because these counties lie within high altitude areas (Jaetzold and Schmidt, 2007). Strains of *R. solanacearum* have been reported in many parts of the world and it affects over 200 plant species in over 50 families (Champoiseau and Momol, 2009).

The findings revealed that farmers used seed of poor quality from their own farms and neighbours and continued planting it year in year out without renewing the seed from certified seed sources. This contributed highly to the problem of bacterial wilt and viruses. In the past,

previous research reported incidence of bacterial wilt at altitudes of 2942 masl which was contributed by factors like source of seeds (Ateka, *et al.,* 2001). Comparatively, Meru County produced more potatoes than the other four counties surveyed with 56% respondents growing this crop. The average farm size under potato production ranged from 0.3 to 0.7 ha for the counties surveyed with Nakuru leading followed by Meru and Kiambu. It is clear that the sizes of the farms in the areas surveyed are relatively small (less than a hectare) and farmers are not able to practice long period rotation of three to four years. The problem of small farm size limits long crop rotation (Lutaladio *et al.,* 1995). Farmers were also replanting poor quality potato seeds in the same farm thereby increasing disease incidence and prevalence.

Most respondents (69%) obtained seed from their own farms, led by Nyandarua with 69% while others obtained seeds from neighbours. Nyeri County had the highest percentage of farmers who obtained seed potato from neighbours. Renewal of seeds was done by a few farmers except Kiambu farmers who obtained clean seed potato from KARLO. Own farm seed potato are usually of poor quality and planted continuously for several years, they show degeneration of tubers which is a result of viruses and virus-like organisms that accumulate in them (FAO, 2010). In Nyandarua, most of farmers did not obtain seed from reliable sources and were not renewing seed and therefore, the incidence of viral diseases was high and disease prevalence was 91%. In Meru, most farmers also obtained seed potato from unreliable sources and did not renew seed and this led to high virus incidence and the disease prevalence was 86%. Nyeri farmers used seed from unreliable sources resulting into high incidence of virus with disease prevalence of 80%. Research conducted in the past showed that PLRV prevalence in Nyandarua (North Kinangop) was 99%, Meru (Central) 91%, Nyeri 91%, and Nakuru (Elburgon) 29% (Gildemacher *et al.,* 2009).

The study indicates that BW was prevalence in all the five counties and the incidence was relatively high. Farmers who used seeds from their own farms had very high incidence of bacterial wilt and viruses. Farmers in Nyandarua County used their own seed (69.3%). These results show that most farmers in the surveyed region do not renew seed and this explains the high incidence and prevalence of bacterial wilt and viral diseases. Lack of adequate certified seed potatoes for planting forces farmers to plant poor quality seeds from their own farms thereby increasing seed-borne diseases leading to high incidences and prevalence of BW and PLRV in these potato production areas. Previous research indicates that 77% of potato farms in Kenya were affected by bacterial wilt (Muthoni *et al.,* 2013; Peeters *et al.,* 2013).

Crop rotation sequence also plays a role in disease prevalence and it can be seen that in Nakuru where potato is rotated with rhodes grass, maize, and other non-host crops, PLRV was low. Previous research shows that planting potato crop from seed produced in the same farm without rotation with none host crop leads to poor quality seed potato due to degeneration of tubers caused by virus disease (FAO, 2010). It was noted that even where rotation was done with non-host crop, the incidence and prevalence of bacterial wilt and potato leaf roll was high and this was due to replanting diseased potato seeds from the same farms. This leads to the spread of seed-borne diseases which increases incidence and prevalence of BW and PLRV in the production areas. Most farmers could not meet the rotation sequence recommended because the farm sizes are small. In summary, use of poor quality seed from farmers or neighbours infected with diseases, lack of renewing seed after two years and using degenerated seed due to diseases led to high prevalence of BW and PLRV. Small farms limiting rotation led to high disease prevalence and incidence and unlike Nakuru using rhodes grass, other farmers rotate with crops that are hosts to aphids which could be compounding the problem.

CHAPTER FOUR

4.0 Evaluation of imported and locally produced potato germplasm for resistance to late blight under field and greenhouse conditions

4.1 Abstract

Potato is one of the important crops in Kenya and it is grown for domestic consumption and as a commercial crop. The crop is affected by many biotic stresses that include bacterial, viral and fungal diseases and insect pests. The objective of this study was to evaluate imported potato germplasm for resistance to late blight disease under Kenyan conditions. The study was conducted at the University of Nairobi, Kabete Campus (UM3), KALRO Genetic Resources Research Institute, Muguga (LH3) and KALRO Njabi-ini in Nyandarua County (UH3). The experiments were carried out from April to June 2015 using randomized complete block design. Kenya Mpya, Sherekea, Shangi, Tigoni, Asante, Nyayo (Local) and Lady Claire, Lady Olympia, Milva, Saprano, Voyanger, Rodeo, Evora, Mantou, Saviola, Sifra, Lady Rosetta, Lady Amarilla, Zafira, Panamera, Arnova, Carolus Challenger and Taurus (imported) were screened for late blight, bacterial wilt and viral diseases in the laboratory before conducting field experiments. The Eucablight key was used to assess potato for late blight infection and data analyzed for area under disease progress curve (AUDPC). The varieties tested showed significant differences in late blight disease resistance. Carolus, Arnova (imported), Kenya Mpya and Sherekea (local) were rated as resistant to late blight after recording no. Kenya Mpya gave yields of 31.7 t/ha, Sherekea, 25.7 t/ha, Carolus 19.5 t/ha and Arnova 18.6 t/ha at different sites indicating positive relationship between yields and resistance to late blight. Other imported cultivars are susceptible to late blight of Kenya.

4.2 Introduction

Potato is an important crop in Kenya and it ranks fourth in the world production, after wheat, maize and rice. The crop is grown for consumption and commercial purposes. Dutch Robijn variety is popular for processing crisps while Tigoni variety is used for frozen chips (Randall *et al.,* 2010). Tigoni variety is also used for chips, French fries, as well as stews or mashed with maize and beans or other pulses to which green vegetables are added (Muthoni *et al.,* 2014). Currently, many varieties of potato are grown in Kenya such as Asante, Tigoni, Kenya Mpya, Sherekea, Shangi, Nyayo and many others.

The crop does very well in mid and highlands areas with altitude between 1500-3000 meters above sea level (masl) with rainfall of more than 600 mm. It requires deep, well drained fertile soils with a pH of 5.5 -6.0 and optimum temperature of 15-18^{0}C. Hilly sites should be avoided and seed size should be 35-45mm in diameter. Pre-sprouting seed is important to achieve early emergence and uniform growth and spraying crop with fungicides and insecticides is recommended. The crop matures in 3-4 months and can yield up to 40 tons per ha (Denis and Mary, 2010).

Potato yield for all varieties is related to resistance to late blight. Sherekea and Kenya Mpya cultivars are tolerant to late blight under good management and Sherekea produces yield of between 40t/ha to 50t/ha and Kenya Mpya 35t/ha to 40t/ha while Purple Gold yields 30t/ha to 35t/ha (Onditi *et al.,* 2012). Other varieties such as Tigoni, Asante, Kenya Mavuno, Kenya Sifa, Kenya Karibu and Kenya Faulu can produce 35 to 45 t/ha (Lung'aho *et al.,* 2006). Sherekea and Kenya Mpya are resistant to late blight, PVY and PLRV and are good for chips, crisps and mashing. Kenya Mpya has a short dormancy period of 75-90 days and matures early in 90 to 105 days (Onditi *et al.,* 2012).

Certified potato tubers have been used for planting to avoid initial inocula and combined with early planting helps the crop to mature before late blight sets in. Farmers use resistant varieties to control late blight and these are obtained from the National Potato Programme at KALRO Tigoni or from the Potato International Centre (CIP). Resistance to late blight depends on a specific environment of potato growing region and therefore it is important to evaluate resistance under local conditions (CIP, 1985). Hilling the crop reduces the inocula by covering fallen leaves carrying *P. infestans* that may continue attacking the crop. Weeding and removal of volunteer plants, destruction of potato remains prevent spores spread by wind. Monitoring programmes help to detect disease early and scouting of potato fields for late blight starts before planting to check presence of source of inocula and continue weekly until harvest time (Agrios, 2004). Fungicides should be sprayed at the underside of the leaves and this commences when the first disease symptoms are noticed (Kabira *et al.*, 2006).

Evaluation of late blight can be done in the morning or at sunrise and when done in the morning it should be completed before 11.00 a.m. when the day is sunny. Disease intensity (severity) is estimated as percentage of leaf area affected and data taken weekly (Lutaladio *et al.,* 1995). Results can be expressed as disease increase curves where data are plotted against time, proportion of leaf area affected in percentage in relation to maximum area to get area under disease progress curve (AUDPC) (CIP, 1985).

Little is known about imported seed potato in Kenya however, due to overwhelming demand for seed potato, the imported seed potato need to be screened for diseases to avoid introduction of quarantine diseases and insect pests into the country. Evaluation for their resistance to late blight under Kenya conditions before they are imported into the country is important.

4.3 Materials and methods

4.3.1 Screening of potato germplasm for resistance to late blight under greenhouse conditions

4.3.1.1 Planting potato in pots

A study was conducted to assess twenty four varieties of imported and local potato germplasm under greenhouse conditions at KEPHIS Muguga in randomized, complete block design (Alan and Scarisbrick, 2001; Mead and Curnow, 1992). There were six compartments in the greenhouses each of them representing a block. Varieties were planted in sterilized pots of 30cm diameter aligned with sterilized gravels (about 19 mm (3/4 inches) avoiding blocking the holes at the base of the pots to prevent waterlogging and pots labeled with variety code and the number of replicate. Pots were filled halfway with sterilized soil (mixture of virgin forest soil, goat and cow manure, about 3.175mm (1/8 inches) gravels at the ratio of 6:3:1) and DAP at the rate of 150g/60kg of soil (150g of DAP mixed with one-wheel barrow of soil)**.** Potato tubers were then planted in pots and every variety was replicated three times. In every pot, only one tuber was planted. Watering plants and maintaining the greenhouses continued following the normal agronomic practices. Plants were top dressed with NPK fertilizer mixed with sterile soil at three weeks after planting at the rate of 150g of NPK in 60kg of sterile soil (150g of NPK to one-wheel barrow of sterile soil) and left to grow up to seven weeks.

4.3.1.2 Isolation of *Phytophthora infestans*

Phytophthora infestans was isolated from young infected leaves of potato. Tubers were selected from Arka variety which is very susceptible to late blight. The selected tubers were of medium size without rots and mechanical damage. They were washed, left to dry, then sterilized by dipping in 70% alcohol (ethanol) for a few seconds and burned off (flaming) as indicated by

CABI, (1983). Using a sterile knife, each potato tuber was cut into about 1cm thick tuber slices so that each slice had two cut surfaces. The outer most part of tubers was discarded. The potato slices were placed on sterile surface in the laminar flow. Lesions of infected leaves with *P. infestans* were cut and placed on the potato wedge made with a sterile knife and potato slices placed in petri dishes each aligned with sterile filter paper. Petri dishes were labeled with accession number of the isolate and date of isolation and then incubated at 18^0C for seven days as per Fry and Shaw (1997). This was followed by sub-culturing of *P. infestans* on Pea Agar medium to get pure cultures of *P. infestans*. On the upper side of the infected tuber slices, sporangia were picked up with a hypodermic needle and placed on Pea Agar without touching potato slices with the hypodermic needle as indicated by Fry and Shaw (1997). Microscopy was used to identify and confirm that the isolated fungus was *P. infestans* before it was used for inoculation of potato plants.

4.3.1.3 Inoculation of potato plants with *P. infestans*

When plants were seven (7) weeks old, they were inoculated with *P. infestans* inocula at 10^4/ml concentrations (Melissa, 2011) with a hand sprayer targeting the underside of the leaves. The inoculum was sprayed to individual plant independently and then the plant was covered with a polythene bag immediately after inoculation. The polythene bags were tacked under the pots and greenhouses were flooded with water to create conducive relative humidity for *P. infestans* growth. Polythene bags were removed after 24 hours and frequent monitoring and scouting for the disease continued until first symptoms of late bright appeared ten days after inoculation. Scoring for the late blight disease infection (lesions on the leaves) was done on the upper and middle leaves for five weeks consecutively and once a week. Five disease scores were recorded.

Intensity of late blight disease attacks was estimated as percentage of leaf area affected using Eucablight key that was adapted from CIP (Colon *et al.,* 2004) (Table 4.1).

Table 4.1: Eucablight key for scoring late blight disease on potato plants at the greenhouse

Severity		Description
0.0%	1	No disease observed
0.1%	2	First sporulation in the green houses
1.0%	3	General light infection about 5-10 lesions /plant
5.0%	4	About 50 lesions /plant; 1 in 10 leaflets affected.
25%	5	Nearly every leaflet infected but plants retain normal form; plants may smell of blight. Greenhouses look green although every plant is affected
50%	6	Every plant is affected and about 50% of the leaf area is destroyed. The greenhouses appear green flecked with brown.
75%	7	About 75% of the leaf area destroyed; Greenhouses appear neither predominantly green nor brown.
95%	8	Only a few leaves on plants, but stems are green.
100%	9	All leaves, stems dead or dying

4.3.2 Evaluation of imported and local potato germplasm for resistance to late blight under field conditions

4.3.2.1 Site selection

Three experimental sites with an area of 22x18m each were selected in the potato growing regions of Njabi-ini, in Nyandarua, Gene bank Muguga in Kiambu and University of Nairobi Kabete Campus (Figure 4.1). The regions lie within the altitude 1848 and 2549 masl with University of Nairobi having an altitude of 1848m, Gene Bank 2087m and Njabi-ini 2549 masl. These sites were selected because they lie within the agroecological zones (AEZs) that favour potato production which are mid and highlands ecological zones lying between 1,500 and 3,000 masl (Lutaladio *et al.,* 1995).

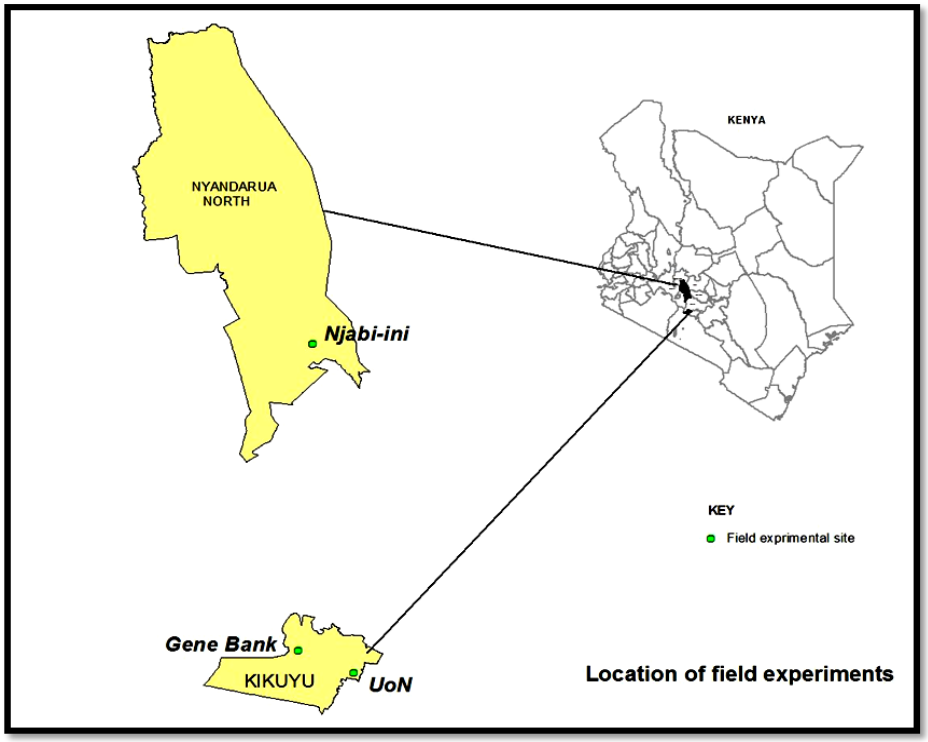


Figure 4.1: Location of field experimental sites

4.3.2.2 Planting potato in the field

Field layout was a randomized complete block design with three replicates of each variety as per the previous research (Mead and Curnow, 1992). Each block comprised of one row of ten tubers

for each variety making a total of thirty tubers in the three blocks. Seven hundred and twenty tubers were used in every site of the experiment. The spacing was 30x75cm leaving a path of 2m between the blocks. A spreader row of a variety sensitive to late blight Arka was planted around each block at 75cm away from the varieties on trial. DAP-18:46:0 fertilizer at the rate of 500kg /ha was used during planting and NPK applied as a source of nitrogen at the rate of 300kg/ha prior to ridging when the crop was about to flower (Randall *et al.,* 2010).

4.3.2.3 Inoculation of plants with *P. infestans* in the field

At Kabete Campus in University of Nairobi, the spreader row was inoculated in the evening to achieve prolonged wetness at night with $1^1/_2$ liters of 10^4 concentrations of *P. infestans* inocula. This was to enhance spread of the disease to the experimental plot at flowering stage. At Genetic Resource Research Institute (GRRI), Muguga and Njabi-ini sites there was enough inocula in the surrounding environment because potato was grown in these areas throughout the year and therefore, natural inocula was sufficient to cause the disease. Previous research indicated that areas where potato was grown continuously natural inocula was enough to spread disease to the experimental plots (Forbes and Corva, 1993, Lung'aho *et al.,* 2008).

4.3.2.4 Scoring for late blight disease in the field

At the appearance of first symptom, scoring for the disease began and data collected once in every week for five weeks following a method adopted from Lutaladio *et al.,* **(**1989**),** Leontine *et al.,* (2004). Five data scores were recorded by estimating visually the percentage leaf area affected by late blight (Table 4.2 & Plate 4). Data Analysis was done to get area under disease progress curve (AUDPC).

Harvesting potato was done four months (120 days) after emergence. During that time, the crop was ready for harvesting as indicated in the previous research (Onditi *et al*., 2012). Weight was using an electrical balance (scale: 1g to 5 kg). Data recorded on weight for the three sites were analyzed to show effects of late blight on yields for both Dutch and local varieties.

Variety: Lady Claire

Plate 4: Symptoms of late blight on potato plants 10 days after inoculation at the greenhouse.

Table 4.2: Field assessment key of late blight caused by *Phytophthora infestans*

SEVERITY	DESCRIPTION
0.0%	No late blight disease observed
0.1%	First sporulation
1.0%	General light infection about 5-10 lesions /plant
2.5%	Late blight present. Maximum 10 lesions per plant
5.0%	About 50 lesions /plant; 1 in 10 leaflets affected.
10%	Plants look healthy, but lesions are easily seen at closer distance. Maximum foliage area affected by lesions or destroyed corresponding to no more than 20 leaflets.
25%	Late blight seen on most plants, about 25% of foliage covered with lesions or destroyed.
50%	Plot looks green however, all plants are affected. Lower leaves are dead, half (50%) of the foliage area is destroyed.
75%	About 75% of the leaf area destroyed; Plot appear green with brown flecks about 75% of each plant affected. Leaves of the lower half of plants are destroyed.
90%	Plot neither predominantly green nor brown, top leaves green. Stems have large lesions.
97.5%	Plot is brown coloured, a few top leaves have some green areas. Most stems have lesions or are dead.
100%	All leaves and stems dead

4.4 Results

4.4.1 Late blight severity under greenhouse conditions

The twenty four varieties (local and imported) that were evaluated for late blight resistance under greenhouse conditions showed variation in terms of susceptibility and resistance. Disease progress was expressed as percentage of foliage with lesions as evaluated at weekly intervals. All imported varieties had high disease intensity/severity (Figure 4.2) with AUDPC value between

500 and 2250, Zafira lead with followed by Arnova at 2200 while Carolus had AUDPC value of 1,500. Local varieties were resistant to late blight with low disease intensity of between 20 and 46 AUDPC values under greenhouse environment. Sherekea had the lowest AUDPC value of 20 followed by Shangi at 36 and Asante 37 respectively. (Figure 4.2).

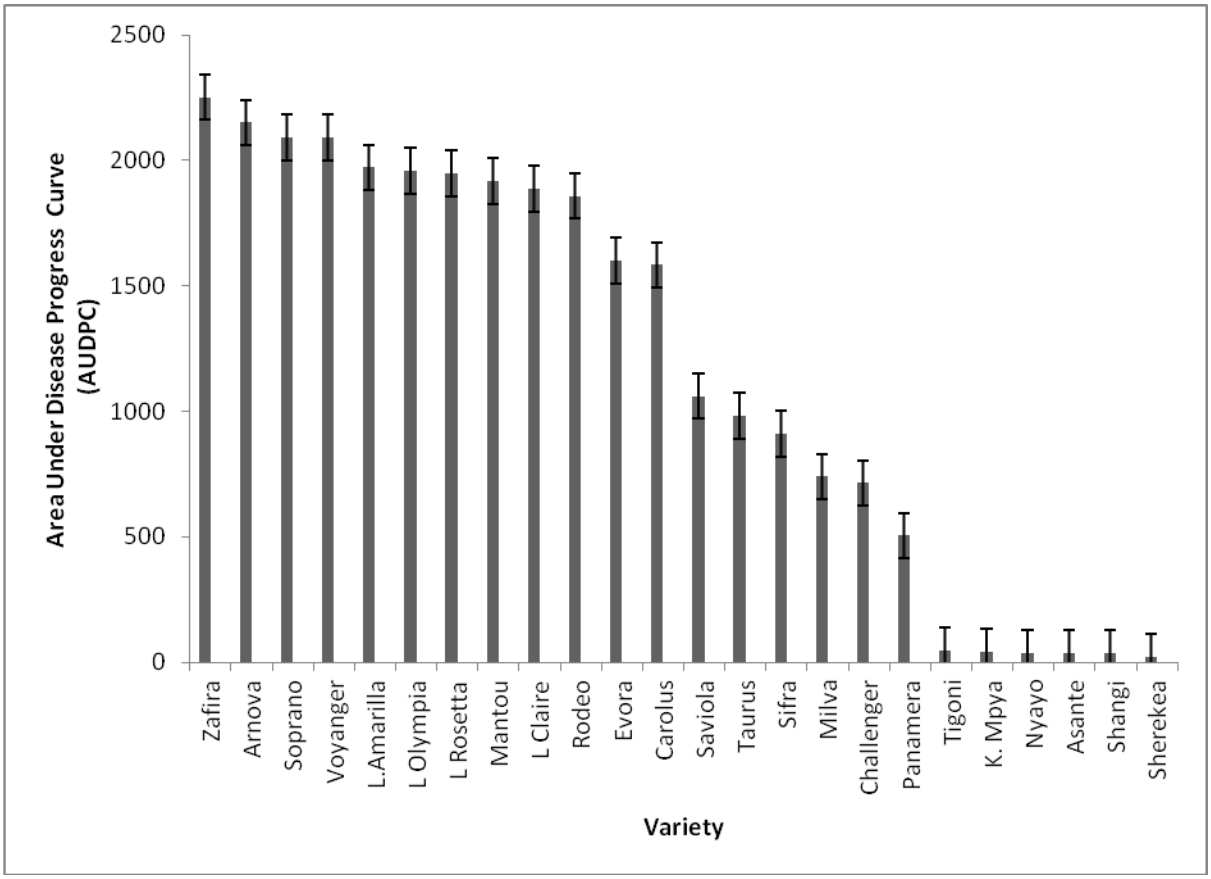
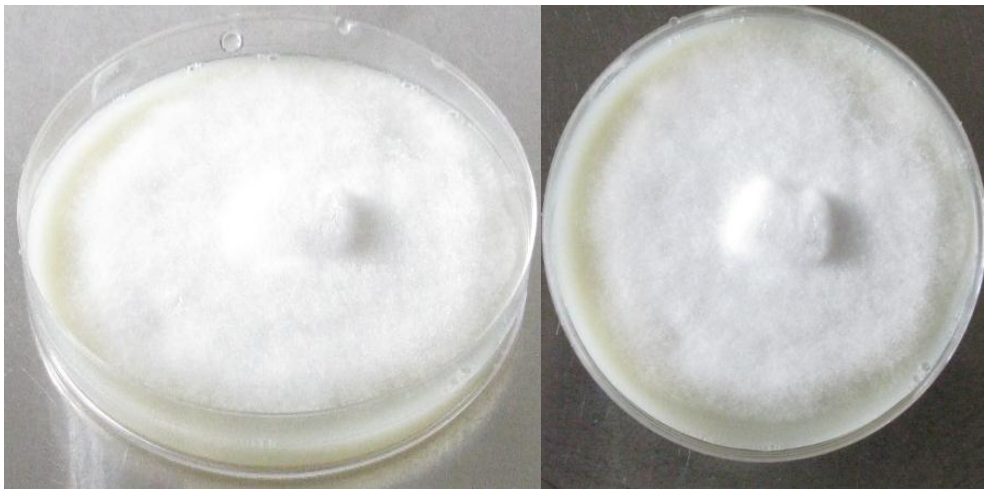


Figure 4.2: Area under disease progress curve for late blight for the various potato varieties under greenhouse conditions at Muguga, Kenya.

Cultures of *P. infestans* which were used to inoculate plants in the greenhouse appeared white on Pea Agar media (Plates 1a, 1b & 2) and mycelia bore sporangia at the tip of the hypha. Observation under the compound microscope showed that sporangia were hyaline, lemon shaped and had papilla (Plate 3).



1a 1b

Plate 1: Cultures of *P. infestans* (1a, 1b) grown on Pea Agar media

Plate 2: *P. infestans* slants

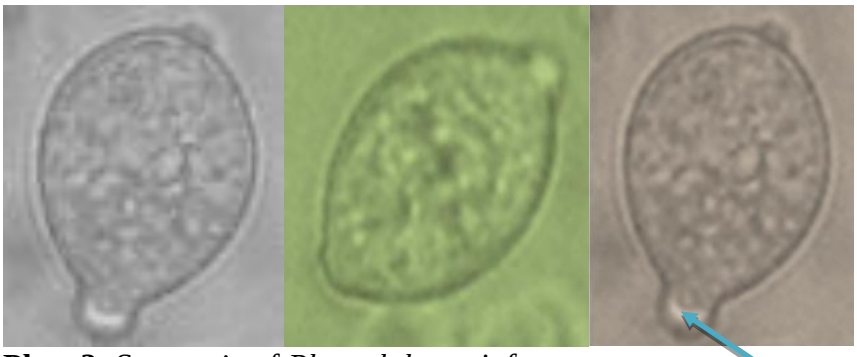


Plate 3: Sporangia of *Phytophthora infestans*

4.4.2 Disease severity in the field

The twenty four varieties (local and imported) evaluated for late blight in the field showed significant difference ($P<0.001$) in terms of susceptibility and resistance. At the University of Nairobi site, all the imported varieties apart from Arnova and Carolus were susceptible to late blight under field conditions compared to the local ones. Local varieties Kenya Mpya and Sherekea also showed high levels of resistance to late blight compared to all the other varieties tested. Cultivar Taurus had AUDPC value of 106 and was in the level of Tigoni, Shangi, Asante and Nyayo with values ranging between 1000 and 1727. Challenger had lower AUDPC value than Asante and Nyayo. All the imported varieties were susceptible to late blight with exception of cultivar Arnova and Carolus. There was significant difference in AUDPC at the University of Nairobi site between Carolus and Claire and Taurus and Claire.

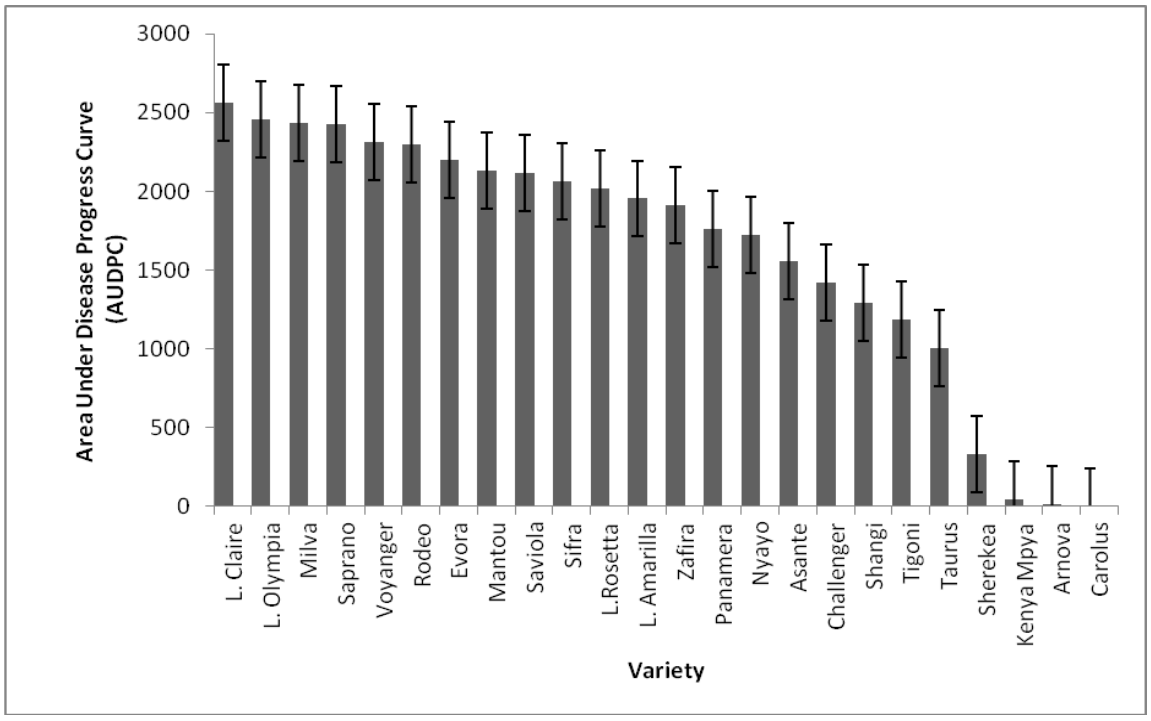


Figure 4.3: Area under disease progress curve for late blight for various potato varieties at the University of Nairobi site.

At the Genetic Resource Research Institute (GRRI) Muguga site, there were significant differences (P<0.001) in late blight resistance among varieties and two Dutch varieties Carolus and Arnova showed high levels of resistance to the disease and their level of resistance was not significantly different from that of the cultivar Kenya Mpya. Sherekea variety also showed high resistance compared to all other varieties both local and imported while Dutch variety Taurus was less susceptible to the disease than Nyayo, Tigoni, Shangi and Asante (Figure: 4.4).

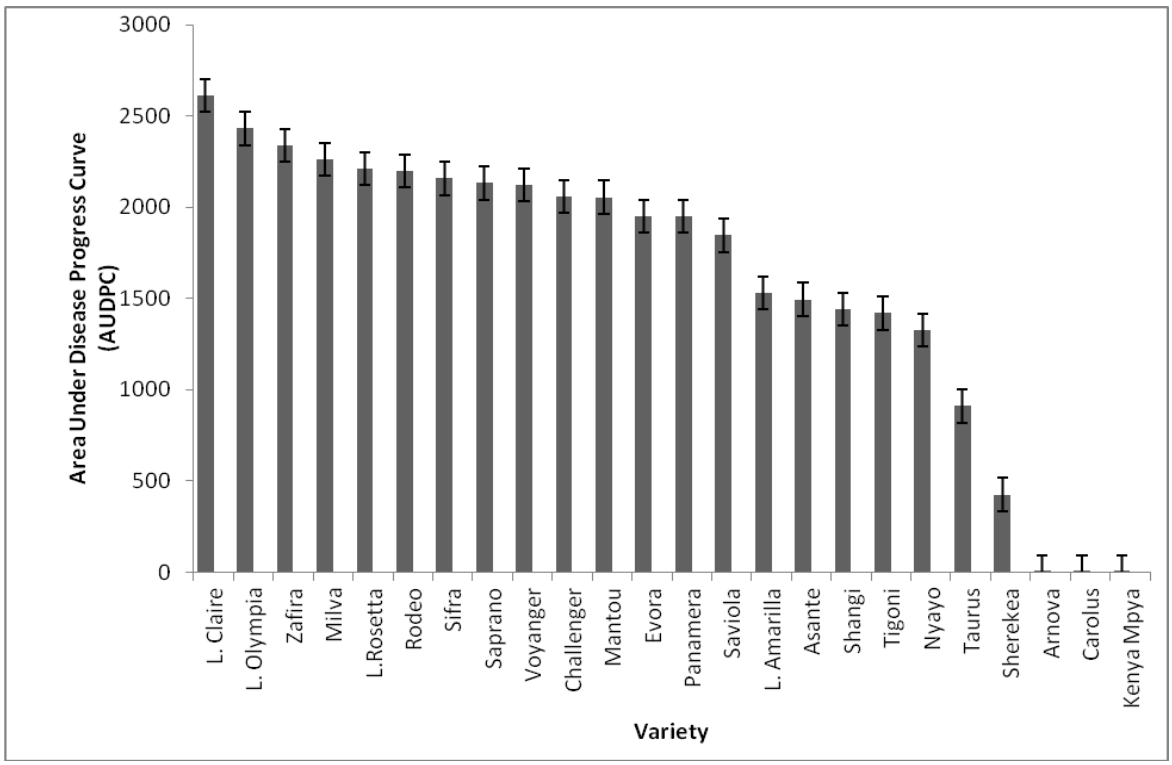


Figure 4.4: Area under disease progress curve for late blight for the various potato varieties at the Gene Bank site, Muguga.

At the Njabi-ini site the varieties showed significant differences in resistance (P<0.001) to late blight. Dutch varieties, Arnova and Carolus level of resistance was not significantly different from the local varieties Kenya Mpya and Sherekea. Varieties Tigoni, Shangi and Nyayo had higher resistance to the disease than the rest of the imported varieties. Taurus performed poorly

at Njabi-ini and local variety Asante could not resist late blight. All the other varieties both local and imported were susceptible to the late blight disease. All the other imported varieties were susceptible to late blight at Njabi-ini site (Figure 4.5).

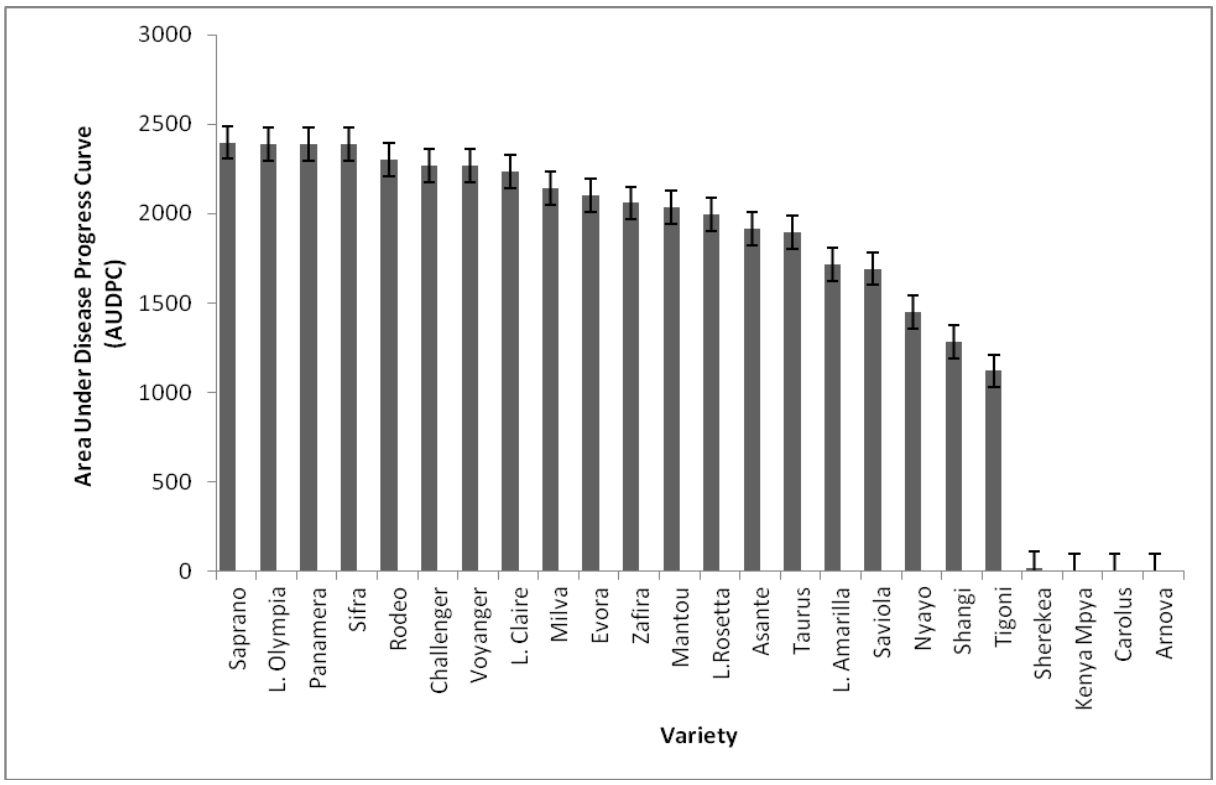


Figure 4.5: Area under disease progress curve for late blight for the various potato varieties at Njabi-ini site

Disease severity among the varieties in the greenhouses and in the field was different where Carolus and Arnova were resistant to the late blight at all sites in the field but, susceptible to the disease at the greenhouse. Other imported varieties were susceptible to late bight both in the field and in the greenhouse. Kenya Mpya and Sherekea cultivars were resistant to late blight in the field and in the greenhouse while other local varieties namely Asante, Nyayo, Tigoni and Shangi were resistant to the disease at the greenhouse but susceptible in the fields at all the sites. There

was no significant difference in AUDPC among all the local varieties under greenhouse conditions (Table 4.3).

Table 4.3: Area under disease progress curve for the late blight from the various varieties at the different sites

	Site			
Variety	University of Nairobi	Njabi-ini	Gene Bank	Greenhouse
Lady Claire	2561 a	2234 abcd	2613 a	1887 c
Lady Olympia	2456 a	2386 a	2433 ab	1960 bc
Saprano	2427 ab	2398 a	2132 cde	2093 abc
Voyanger	2316 abc	2269 abc	2124 cde	2093 abc
Rodeo	2300 abc	2304 ab	2200 bcde	1859 c
Zafira	1911 abcdef	2059 bcde	2339 bc	2252 a
Mantou	2129 abcd	2036 cde	2055 def	1919 bc
L.Rosetta	2018 abcde	1995 de	2211 bcd	1948 bc
Evora	2199 abcd	2100 bcde	1952 ef	1601 d
Milva	2432 ab	2141 abcde	2265 bcd	740 fgh
Sifra	2060 abcde	2386 a	2158 cde	910 efg
Lady Amarilla	1954 abcdef	1715 f	1532 g	1972 bc
Challenger	1419 efgh	2269 abc	2059 def	715 gh
Saviola	2118 abcd	1692 fg	1846 f	1061 e
Panamera	1763 bcdefg	2386 a	1951 ef	505 h
Taurus	1006 hi	1896 ef	976 h	983 ef
Asante	1559 defgh	1913 ef	1495 g	37 i
Nyayo	1727 cdefg	1447 gh	1325 g	38 i
Shangi	1290 fgh	1283 hi	1441 g	36 i
Tigoni	1184 gh	1120 i	1420 g	46 i
Arnova	12 j	2 j	1 j	2151 ab
Carolus	2 j	3 j	1 j	1583 d
Sherekea	333 ij	17 j	426 i	20 i
Kenya Mpya	45 j	8 j	0 j	42 i
Mean	1634	1669	1620	1186
LSD (P 0.05)	688.2	26 3.6	256.9	254.7
CV (%)	25.6	9.6	9.6	18.8

NB: Variety means with the same letters along the columns have no significant difference in mean disease severity

Mean disease severity for every potato variety at different sites in the field was evaluated and comparison of means difference for each variety across the different sites made. Varieties Arnova,

Carolus and Milva (imported) showed significant difference in means of disease severity. Local varieties Sherekea and Nyayo also had significant difference in means of disease severity and therefore, there was site interaction among the varieties. Other local and imported varieties had no significant difference in disease severity mean indicating no site interaction among them (Table 4.4).

Table 4.4: Area under disease progress curve for the late blight from the various varieties at the different sites

Variety	Site			Mean	LSD (p=0.05)	CV(%)
	Gene bank	Nairobi	Njabi-ini			
Arnova	0.82b	11.55a	2.10b	4.8	1.436	13.1
Asante	1495a	1559a	1913a	1655.7	655.9	17.5
Carolus	0.82b	2.45a	2.68a	2.0	1.449	32.2
Challenger	2059a	1419a	2269a	1915.7	1654.5	38.1
Evora	1952a	2199a	2100a	2083.7	438.6	9.3
K. Mpya	0.20a	44.80a	8.40a	17.8	47.23	117
L. Amarilla	1532a	1954a	1715a	1733.7	416.7	10.6
L. Claire	2613a	2561a	2234a	2469.3	382.8	6.8
L. Olympia	2432a	2456a	2386a	2424.7	227.2	4.1
L. Rosetta	2211a	2018a	1995a	2074.7	244.9	5.2
Mantou	2055a	2129a	2036a	2073.3	605.8	12.9
Milva	2264b	2432a	2141c	2279.0	107	2.1
Nyayo	1325b	1727a	1447b	1499.7	232.4	6.8
Panamera	1951a	1763a	2386a	2033.3	847.9	18.4
Rodeo	2200a	2300a	2304a	2268.0	277.9	5.4
Sangi	1441a	1290a	1283a	1338.0	355	11.7
Saprano	2132a	2427a	2398a	2319.0	429.6	8.2
Saviola	1846a	2118a	1692a	1885.3	493.9	11.6
Sherehekea	426a	333a	17b	258.7	100.9	17.2
Sifra	2158a	2060a	2386a	2201.3	439.8	8.8
Taurus	910a	1006a	1896a	1270.7	1880.4	65.3
Tigoni	1420a	1184a	1120a	1241.3	708.8	25.2
Voyanger	2124a	2316a	2269a	2236.3	236.1	4.7
Zafira	2339a	1911a	2059a	2103.0	530.2	11.1

NB: Variety means with the same letters across the sites have no significant difference in mean disease severity

4.4.3: Yield of potato varieties evaluated for LB resistance under field conditions

At the University of Nairobi site, significant differences (P<0.001) in yields were observed among all the varieties tested. The Dutch varieties, Carolus with mean yield of 18.3 t/ha and Arnova 18.6 t/ha were leading in yield performance followed by local variety Sherekea with 17.3t/ha and Shangi (17.2t/ha). Kenya Mpya with a yield of 9.0 t/ha. Yield of Kenya Mpyavariety had no significant difference from yields of imported varieties Voyager and Saviola and local ones Nyayo and Tigoni. Asante, Nyayo and Tigoni yielded higher than the rest of the imported varieties. The rest of the imported varieties had mean yield ranging between 0.8 t/ha and 8.9 t/ha, respectively (Fig: 4.6; Table 4.5).

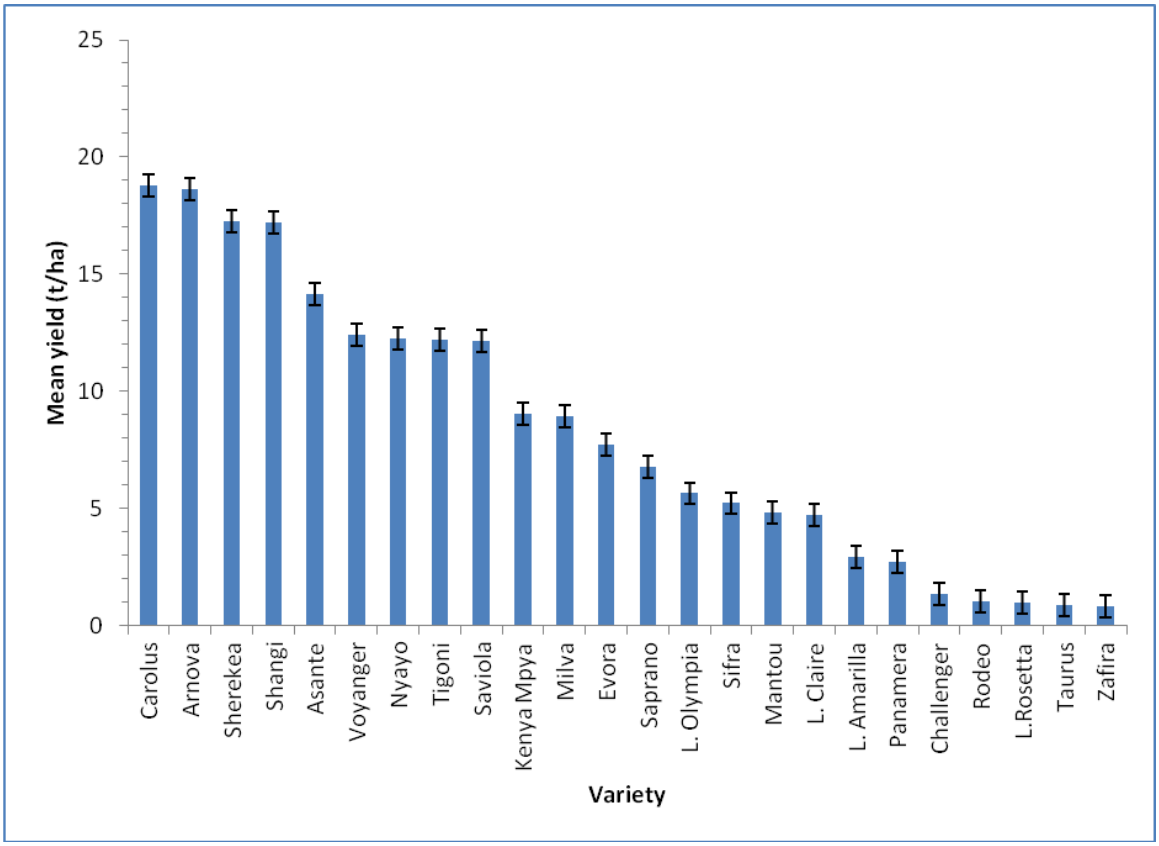


Figure 4.6: Mean yields of tubers in tons per hectare for the varieties at the University of Nairobi site

At the GRRI site in Muguga, significant differences in yields (P<0.001) was observed among the varieties tested. Local variety Sherekea yielded the highest with a mean yield of 22.1 t/ha among all the varieties tested followed by imported variety Arnova (16.3t/ha), Carolus (12.2 t/ha) and local variety Kenya Mpya (11.5 t/ha). Nyayo, Tigoni, yielded an average of 6.5 t/ha, and 8.9 t/ha, respectively. The rest of the imported varieties had mean yields between 1 to 3 t/ha and were not significantly different (Fig: 4.7: Table 4.5).

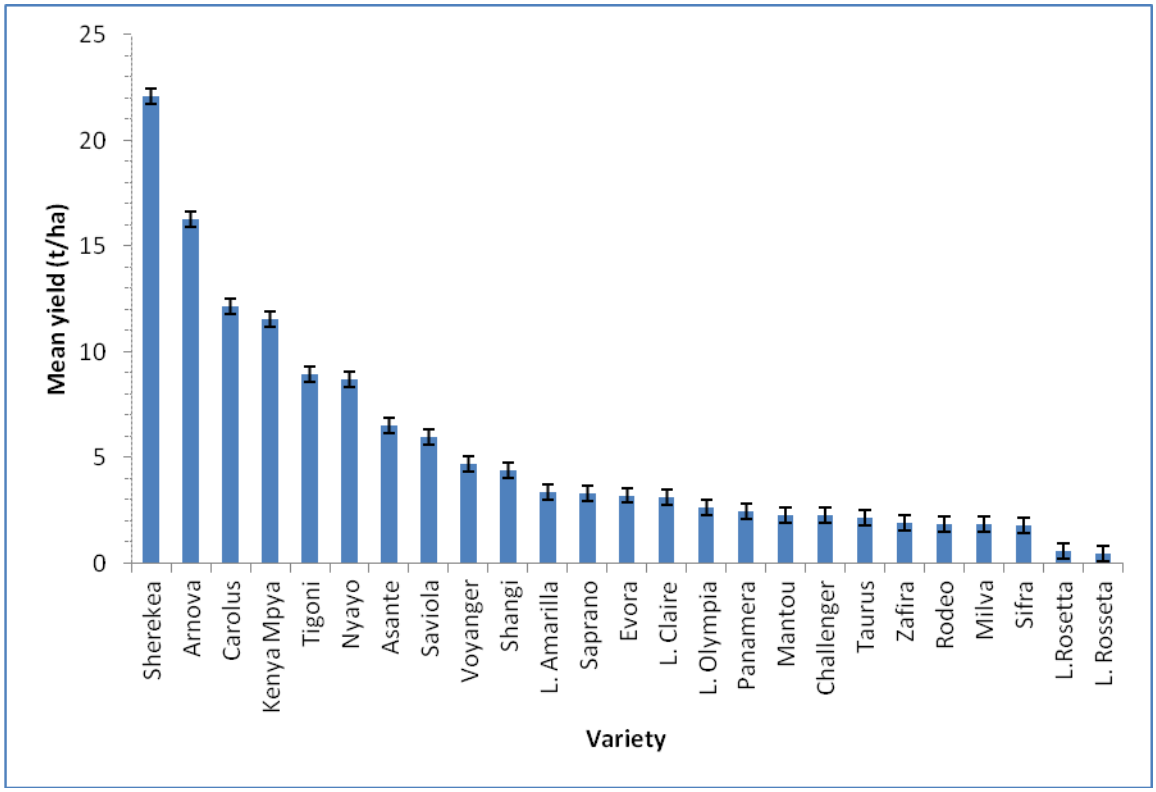


Figure 4.7: Mean yields of tubers in tons per hectare for the varieties at Gene Bank site in Muguga.

At Njabi-ini site which is in the high altitude area, tested varieties showed significant differences (P<0.001) in yields. Kenya Mpya had the highest mean yields of 31.7 t/ha followed by Sherekea with 25.7 t/ha. The two imported varieties, Carolus (19.5 t/ha) and Arnova (15.9 t/ha) had high

mean yields compared to the rest of local and other imported varieties. The rest of the varieties both local and imported performed poorly with mean yields of between 0.4 to 2.8 t/ha. Shangi yielded lowest with mean yields of 1.6 t/ha. (Figure: 4.8; Table 4.5).

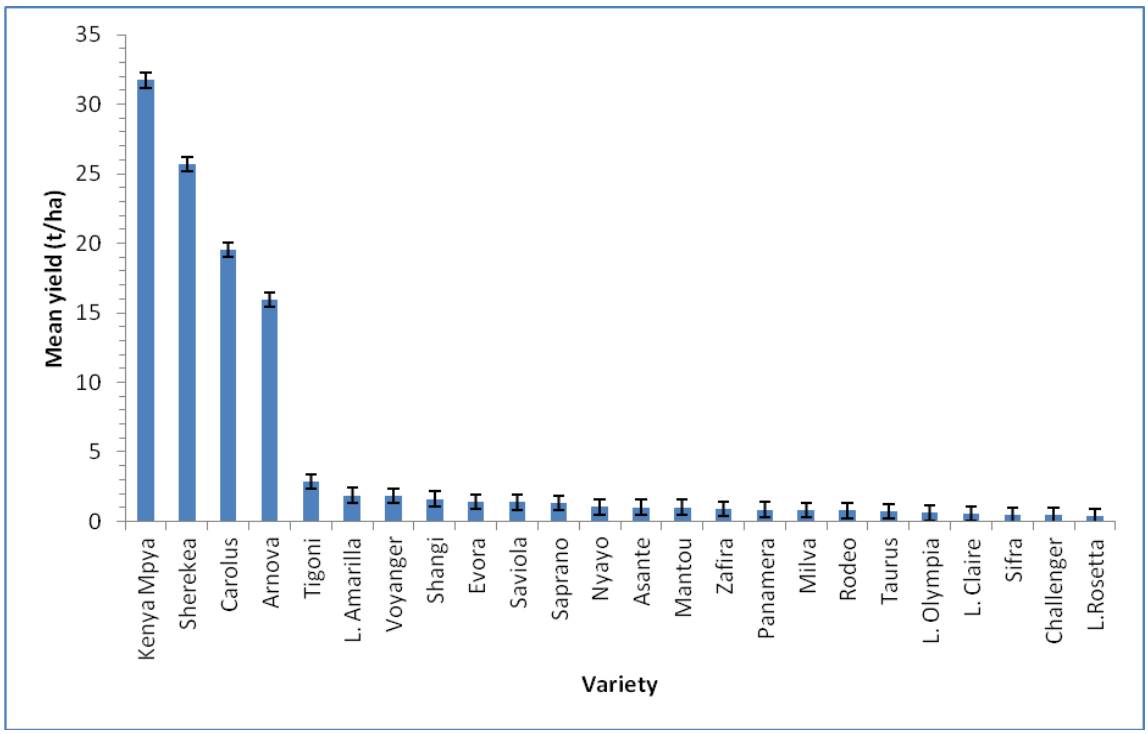


Figure 4.8: Mean yields weight of tubers in tons per hectare for the varieties at Njabi-ini site.

Yields of most of the imported potato varieties were below the average potato yield production by the small scale farmers in Kenya. The average potato production is 7.7t/ha (Janssen, 2014). Arnova variety had the highest mean yields at the University of Nairobi site while Carolus variety had highest mean yields at Njabi-ini. Kenya Mpya and Sherekea varieties gave highest mean yields at Njabi-ini site and lowest at the University of Nairobi site. Shangi had highest yields at UON and low yields at Njabi-ini and Gene bank. There was a wide difference in mean yields among the susceptible local varieties from the three sites (Table 4.5).

Table 4.5: Mean yields in t/ha for various potato varieties at the different sites

Variety	Sites			
	University of Nairobi	**Gene Bank**	**Njabi-ini**	**Mean yields**
Kenya Mpya	9.0 de	11.5 cd	31.7 a	21.69a
Carolus	18.8 a	12.2 c	19.5 c	17.43b
Arnova	18.6 a	16.3 b	15.9 c	16.96b
Sherekea	17.3 ab	22.1 a	25.7 b	16.83b
Shangi	17.2 ab	4.4 fgh	1.6 d	8.00c
Asante	14.1 bc	6.5 ef	1.0 d	7.74c
Voyanger	12.4 cd	4.7fgh	1.8 d	7.32c
Nyayo	12.2 cd	8.7 de	1.0 d	7.22c
Tigoni	12.2 cd	8.9 de	2.8 d	6.49c
Saviola	12.1 cd	6.0efg	1.4 d	6.30cd
Milva	8.9 de	1.8 hi	0.8 d	4.11de
Evora	7.7 ef	3.2 ghi	1.4 d	3.86de
Saprano	6.7 efg	3.3 ghi	1.3 d	3.79de
L. Olympia	5.6 efg	2.6 hi	0.6 d	2.96ef
Sifra	5.2 efgh	1.8 hi	0.5 d	2.80ef
Mantou	4.8 fghi	2.3 hi	1.0 d	2.72ef
L. Claire	4.7 fghi	3.1 ghi	0.5 d	2.71ef
L. Amarilla	2.9 ghi	3.4 ghi	1.9 d	2.50ef
Panamera	2.7 ghi	2.5 hi	0.8 d	2.01ef
Challeger	1.3 hi	2.3 hi	0.5 d	1.36f
Rodeo	1.0 i	1.9 hi	0.8 d	1.26f
L. Rosetta	1.0 i	1.0 i	0.4 d	1.21f
Taurus	0.9 i	2.3 hi	0.7 d	1.21f
Zafira	0.8 i	1.9 hi	0.9 d	0.78f
Mean	**8.27**	**5.61**	**4.78**	
LSD (P≤0.05)	**4.05**	**3.01**	**4.53**	
CV (%)	**4.3**	**5.1**	**7.8**	

NB: Mean yields having the same letters along the columns are not significantly different.

Mean yields of each Variety were evaluated across the different sites to determine the influence of sites interaction. There was no significant difference in yields among the imported varieties apart from Carolus, Evora, L. Claire, L. Olympia, Mantou, Milva, Soprano, Saviola, Voyanger and Sifra. Mean yields of locally produced varieties namely Kenya Mpya, Asante, Nyayo,

Shangi and Tigoni had significant differences at all the sites. Sherekea (local) and Arnova (imported) varieties had high mean yields which were not significantly different. Site interaction did not influence means of Sherekea and Arnova varieties (Table 4.6).

Table 4.6: Mean yields in t/ha for potato varieties at the different sites

Variety	Site			Mean	LSD (P=0.05)	CV(%)
	Gene bank	Nairobi	Njabi-ini			
Arnova	16.30a	18.65a	15.93a	17.0	5.30	13.8
Asante	6.51b	14.13a	1.02c	7.2	5.48	33.5
Carolus	12.18b	18.76a	19.54a	16.8	4.92	12.9
Challenger	2.27a	1.34a	0.47a	1.4	2.01	65.0
Evora	3.22b	7.73a	1.38b	4.1	3.15	33.8
Kenya Mpya	11.50b	9.00b	31.70a	17.4	17.31	43.8
L. Amarilla	3.38a	2.92a	1.87a	2.7	4.48	72.6
L. Claire	3.14a	4.72a	0.55b	2.8	1.61	25.3
L. Olympia	2.62ab	5.64a	0.60b	3.0	3.53	52.8
L. Rosetta	1.01a	0.97a	0.35a	0.8	0.68	38.3
Mantou	2.28b	4.83a	1.01b	2.7	1.53	25.0
Milva	1.83b	8.92a	0.82b	3.9	3.90	44.6
Nyayo	8.69b	12.24a	1.04c	7.3	1.57	9.4
Panamera	2.46a	2.72a	0.85a	2.0	2.69	59.2
Rodeo	1.85a	1.00a	0.77a	1.2	1.68	61.2
Sangi	4.40b	17.20a	1.60b	7.7	8.34	47.5
Saprano	3.29b	6.75a	1.34b	3.8	2.84	33.1
Saviola	5.96b	12.12a	1.38c	6.5	3.71	25.2
Sherehekea	22.10a	17.30a	25.70a	21.7	13.03	26.5
Sifra	1.79ab	5.22a	0.48b	2.5	3.58	63.2
Taurus	2.18a	0.89a	0.70a	1.3	2.03	71.1
Tigoni	8.94a	12.19a	2.85b	8.0	3.58	19.8
Voyanger	4.68b	12.40a	1.81c	6.3	2.09	14.6
Zafira	1.91a	0.83a	0.89a	1.2	1.66	60.5

NB: Means represented with the same letter across the sites have no significant difference.

4.5 Discussion

Potato production is suited to agroecological zones lying between 1,200 to 3,000 masl (Lutaladio *et al.,* 1995). The research was conducted in the greenhouse at KEPHIS, Muguga and in the field at three sites namely Nyandarua at Njabi-ini, Kikuyu at Gene Bank Muguga and Nairobi University at Kabete Campus which are within the potato production zones.

Under greenhouse conditions, local varieties Tigoni, Kenya Mpya, Nyayo, Asante, Shangi and Sherekea had very low AUDPC values, Cañizares and Forbes (1995) reported low AUDPC values to indicate the possession of minor resistance genes *P. infestans*. *P. infestans* and potato interact according to the gene-for-gene model (Lee *et al.,* 2001). These are 11 R-genes, which suggest the presence of 11 corresponding virulence or virulence factors in *P. infestans*, and genetic analyses on both host and pathogen have been performed to confirm the gene-for-gene model in this pathosystem (Song *et al.,* 2003). All the imported varieties showed high AUDPC values up to 2250 under greenhouse environment implying that imported germplasms are susceptible to the late blight of Kenya under greenhouse conditions.

Under field conditions, the imported cultivars Carolus and Arnova had very low AUDPC values indicating that they have high resistance to the disease. Carolus and Arnova had very high resistance to late blight with AUDPC of 1.0 under field conditions and can be compared with two local varieties Sherekea and Kenya Mpya which were not significantly different. Previous research indicates that several potato varieties were tested for resistance to late blight and released to farmers. In 1998, Tigoni and Asante were released and in 2002, Kenya Sifa, Kenya Karibu, Kenya Faulu and Kenya Mavuno were also released (Lung'aho *et al.,* 2006). In 2012, three varieties highly resistant to late blight Sherekea, Kenya Mpya and Purple Gold were

released and these could not compare with all other locally bred varieties like Asante and Tigoni (Onditi *et al.,* 2012).

In this research, Sherekea and Kenya Mpya were found to be the best in resistance to late blight and yields performance and their yields were relatively high compared to the rest of the varieties. This indicates that the two varieties are resistant to late blight disease and are high yielding. Research in the past had reported that Sherekea can give yields of between 40 to 50 t/ha and does well at altitudes of 1800-3000 masl while Kenya Mpya yields 35 to 45 t/ha and performs well at altitude of 1400-3000m when all other factors are held constant. In the past, Sherekea was also found resistant to viruses PVY and PLRV and Kenya Mpya to PVY and PVX (Onditi *et al.,* 2012). In this study, it was noted that mean yields for Kenya Mpya were significantly different at all the sites while Sherekea variety gave mean yields that were not significantly different at the various sites. Mean yields for Kenya Mpya and Sherekea increased with increase in altitude and they are known to do well at altitude between 1400-3000m as per the previous research (Onditi *et al.,* 2012).

It was also found that late blight disease was influenced by long periods of wetness and different ranges of temperature. The disease intensity increased with decrease in temperatures and increase in rainfall. It was highest at Njabi-ini with an average temperature of 13^0C and annual rainfall of 2,300mm. All the varieties both local and imported were susceptible to the disease apart from Carolus, Arnova, Kenya Mpya and Sherekea cultivars. Disease intensity was also high at the GRRI Muguga where average temperature was 18^0C and annual rainfall of 1,900mm. Disease intensity and incidence was lower at University of Nairobi where average temperature was 22^0C and rainfall 1,250mm than Muguga and Njabi-ini.

Previous research indicates that potato yield is affected by diseases and pests among other parameters. Various varieties yield differently depending on individual variety resistance to diseases. Work done in the past showed that Sherekea variety gives yields of between 45 to 50 t/ha and Kenya Mpya 40 to 45t/ha (Onditi *et al.,* 2012). This study showed that Sherekea, Kenya Mpya and Shangi are high yielding and resist late blight differently in different areas. Sherekea performed well in terms of yields at the three sites with highest mean yields (25.7 t/ha) at Njabi-ini. Shangi variety had highest yields at University of Nairobi site (17.2 t/ha) which is 1848 masl and is warmer than GRRI Muguga and Njabi-ini with altitude of between 2087 and 2549 masl. Kenya Mpya performance gave highest mean yields (31.7 t/ha) at Njabi-ini and lowest at the University of Nairobi.

The study indicates that performance of the two imported varieties Arnova and Carolus was higher at various sites than the other local and Dutch varieties except Sherekea and Kenya Mpya. These two varieties Carolus and Arnova were more adaptable to climatic conditions in Kenya than all the other imported varieties. Previous research indicates that potato cultivars adaptability to climatic conditions is reflected in the average yield of the crop (Mitiku, *et al*., 2015). Carolus and Arnova cultivars can compare with Sherekea and Kenya Mpya which gave very high yields and resisted late blight well at the different experimental sites. The four varieties, two local (Sherekea and Kenya Mpya) and two imported (Arnova and Carolus) showed high degree of resistance to late blight under field conditions in Kenya. Previous research indicates relationship between yields and late blight resistance for various varieties (Tahtjarv, *et al*., 2013). Arnova had highest mean yield (18.6 t/ha) at the University of Nairobi and the means yields from all the sites were not significantly different. Carolus variety had the highest mean yield (19.5 t/ha) at the University of Nairobi and mean yields were significantly different at all the sites. The study also

revealed that these two imported varieties gave high yields in areas with low rainfall and temperatures of between 20^0C and 22^0C.

Mean yields of Sherekea were not significantly different at all the sites but means yields of Kenya Mpya were significantly different. Local varieties Sherekea and Kenya Mpya gave higher yields at the high altitude area of Njabi-ini with high rainfall and low temperatures of as low as 13^0C implying that they are cool climate cultivars. Previous research indicates that potato grows well in cool climate with temperature ranging from 10^0C to 23^0C and the optimal temperature is between 15^0C - 25^0C (FAO, 2015). The crop also does very well in the highlands at 1500-3000 m above sea level with rainfall of more than 600 mm where soils are deep, well drained and fertile with a pH of 5.5 - 6.0 (Kabira *et al.,* 2006).

CHAPTER FIVE

5.0 Evaluation of imported, local certified and informal potato seed for seed-borne infections

5.1 Abstract

In Kenya, seed potato is produced through various production systems including formal and informal seed multiplication systems. Diseases are among the constraints to potato production and the major diseases are bacterial wilt caused by *R. solanacearum*, late blight caused by *P. infestans* and viral diseases. The objective of this study was to assess potato seed from different production systems for pathogenic diseases transmitted through seed tubers. Seed tubers were sampled from certified seed system of KALRO at Tigoni Potato Research Centre and Njabi-ini, registered seed merchants at Kisima and Turi farms, ordinary farmers and imported potato seed. Potato germplasm was screened using both serology and quick diagnostic kit (conventional) methods. Nitrocellulose membrane- Enzyme Linked Immuno-Sorbent Assay (NCM-ELISA) was used for bacterial wilt screening while Double Antibody Sandwich-Enzyme Linked Immuno-Sorbent Assay (DAS-ELISA) kit by Centre for International Potato (CIP) was used in the detection of viruses. Results were confirmed using FORSITE quick diagnostic kits from Central Laboratory U.K. for detection of bacterial wilt and viruses. The results showed that certified seed potato from KALRO Tigoni and Njabi-ini, imported seed potato and registered seed merchants Kisima and Turi farms were free from bacterial wilt and viruses indicating that seeds were produced in disease free sites. Seed potato from small scale farmers' production system were infected by viruses and bacterial wilt implying that they were not produced from clean seeds and areas of production were contaminated by seed borne diseases namely bacterial wilt and viral diseases.

5.2 Introduction

Potato is attacked by many disease pathogens including bacteria, viruses and fungi. The major seed-borne diseases are bacterial wilt, late blight and viral diseases. Bacterial wilt (brown rot) caused by **(***R. solanacearum***),** affects more than 30 plant species and the most susceptible are potato, tomato, eggplant, pepper and groundnut. The disease has no known chemical control measure. Shortage of high-quality seed and lack of farmer awareness on proper agronomic practices is a major factor in disease spread. Infected seed tubers, crop residues, contaminated surface water and soil adhering on shoes and tools spread the disease (Randall *et al.,* 2010). The disease causes wilting followed by drooping of leaves and death of plants. When a wilted plant is cut and squeezed, a white mass of bacteria oozes out indicating presence of bacterial wilt disease. Latent infection is detected through techniques such as polymerase chain reaction (PCR) (Pastrik *et al.,* 2000) and Serological tests. ELISA is the most commonly used serological test. Other methods of detection include use of media like Semi selective Media South Africa (SMSA) and quick diagnostic kit for *R. solanacearum* detection (Pastrik *et al.,* 2000). Bacterial wilt is known to cause yield loss of potato through wilting and this loss continues during storage. The disease is estimated to affect three million farm families which accounts for about 1.5 million hectares (Ateka *et al.,* 2001). In Kenya, several reports indicate an increase in incidence of brown rot of potato due to build-up of the disease in the potato growing zones (Ateka *et al.,* 2001, Kinyua *et al.,* 2014).

Viral diseases are also important in potato production and are associated with degeneration and reduction of quality of planting materials. These viral diseases include potato mosaic disease, potato leaf roll (PLRV), potato virus Y (PVY), potato virus X (PVX), potato virus A (PVA), potato virus S (PVS) and potato virus M (PVM) (Were *et al.,* 2013). All these diseases are

transmitted by beetles, aphids, leaf hoppers and white flies respectively. Aphids transmit viral diseases namely PVY, PVA, PVM, PVS and Potato Mosaic virus. Viruses PVX, PVS, PVY are transmitted by vectors and infected tubers. Potato leaf roll virus and potato mosaic viruse are the main causes of seed degeneration. It is important to use clean seed to avoid high potato yield loss (Randall *et al.,* 2010). Potato virus Y (PVY) is transmitted by aphids and spread through infected tubers and can cause yield losses alone or in combination with other viruses such as PVA, PVX or PVS (Mary *et al.,* 2005). Other diseases of potato are early blight (*Alternaria solani*), black scurf (*Rhizoctonia solani*), blackleg (*Erwinia carotovora*) but these are of minor importance in potato production (Denis and Mary, 2010).

Major diseases of the crop are bacterial wilt caused by *R. solanacearum*, late blight and viruses (Gildemacher *et al.,* 2009). In order to meet the demand for seed potato in the country, the Kenya government made an agreement with the Netherlands government on importation of potato into the country (KEPHIS, 2011; MOA, 2005). This made it necessary to conduct a study to determine the level of disease infection in imported and locally produced seed potato.

5.3 Materials and methods

5.3.1 Screening of bacteria wilt (*Ralstonia solanacearum*) (NCM-ELISA kit CIP, 2001).

Two hundred potato tubers were sampled randomly from a consignment of each variety. The tubers were washed and disinfected with 1% sodium hypochlorite. Vascular rings of approximately 0.5g each were removed from of sample of 25 tubers, placed in polythene bags and weighed. Sterile citric extraction buffer (2ml per gram of tuber tissue) was added to the sample crushed and put in an eppendorf tube. Enrichment media (SMSA broth) was added to the eppendorf tube containing potato tuber extract and incubated for 48 hrs at 30^0C. Dot- blotting

was done on Nitro-cellulose membrane (NCM) followed by Serology (NCM-ELISA) involving the following steps: Blocking, Rs- antibody binding complex / conjugated goat anti-rabbit antibodies and colour development which is an enzyme reaction (CIP, 2001).

5.3.1.1 Sample extraction from tubers for *Ralstonia solanacearum* detection

Potato tubers (25 tubers per sample for 16 replicates) of both imported and local varieties were washed separately with tap water to remove soil and disinfected using sodium hypochlorite (NaOCl) solution 1% concentration by dipping potato tubers for 5 minutes. Potato tubers were left to dry on sterilized paper (serviette). A thin slice was cut at the stolon end with a knife sterilized by flaming every time after cutting each sample. Sterilized cuticle remover was used to remove small pieces of the vascular ring approximately 2mm wide and 1mm deep and about 0.5g per ring from a sample of 25 potato tubers replicated 16 times. The tuber pieces were put in a plastic bag for each sample and weighed. Sterile citric extraction buffer 2ml per gram of tuber tissue was added to tuber pieces in the plastic bag and crushed using a wooden pin. The plastic bag was placed vertically on crushed ice for not more than 1 hour to avoid oxidation of the phenols. All the samples were enriched using enrichment broth (SMSA) following a procedure by Elphinstone *et al.,* (1996).

5.3.1.2 Semi selective Media South Africa (SMSA) for *R. solanacearum* detection

SMSA medium was prepared using 10g Bacto peptine, 5ml glycerol, and 1g casamino acid in a liter of distilled water. The mixture was autoclaved for 15 min at a pressure of 121 pounds per square inch (psi) and at 115^0C. The antibiotic solutions were prepared using 10ml of 1% polymyxin B sulphate (100mg/l, (10ml of 1% cycloheximine (100gm/l) optional), 2.5ml of Bacitracin (25mg/l), 500µl of 1% penicillin G (0.5mg/l), 500µl of chloramphenicol (5mg/l),

500µl of 1% crystal violet (5mg/l) and 5ml of 1% 2, 3, 5- triphenyl tetrazolium chloride (TZC; 50mg/l). The solutions of antibiotics were sterilized by filtering using millipores which are sterile filters. SMSA medium was cooled at 50^0C and antibiotic solutions added to the medium (per liter).

5.3.1.3 Sample enrichment

One eighty (180) ml of distilled sterile water was added to 20ml of SMSA enriched broth to dilute it. Five hundred microliter (500µl) (0.5ml) of diluted SMSA broth was dispensed in sterile 1.5 ml Eppendorf. Then 500µl of tuber extract was added to 500µl of SMSA broth using 1000µl micro-pipette with sterile tips. The mixture was incubated for 48 hours at 30^0C with constant agitation by use of an incubation shaker. The mixture was stored at 20^0C after 48hours of incubation until the following day when samples were put on the membrane (dot-blotting).

5.3.1.4 Dot blotting

The content of TBS buffer provided was dissolved in 997.5 ml of distilled water and mixed thoroughly. The pH was adjusted to 7.5 by drop wise addition of hydrochloric acid (HCl) solution up to 2.5ml. Thirty millimeters (30ml) of TBS (Buffer pH 7.5) was poured in a plastic box and the dry membrane was wetted by slowly sliding it into the buffer solution avoiding formation of air bubbles and left to stand for 5 minutes. To apply the sample on the membrane, two sheets of dry filter paper were placed on the laboratory bench and two filter papers humidified with TBS (Buffer) were placed on top of the dry filter papers. The wet membrane was put on the filter paper avoiding formation of the air bubbles until the liquid on the surface of the membrane was absorbed. A clean sterile glass tube was rolled on the membrane to ensure

good contact between the membrane and the filter papers. This allowed the sample to be absorbed by capillary action without expanding the membrane. Twenty microliters (20µl) of the enriched tuber extract was put slowly on the membrane using a micro-pipette holding the pipette vertically and allowing a slight contact with the pipette end on the membrane so that the drop made contact. This was to avoid the drop to fall on the membrane which would expand a lot making the dot not uniform. The membrane was transferred to a dry filter paper using forceps and left to dry for 60 minutes. A code was written with a pencil on the lower left hand corner to help in identification of the samples. Then the membrane was used in serology test.

5.3.1.5 Serological test

Serological test carried out involved blocking, Rs antibody binding and colour development which is an enzymatic reaction. Blocking buffer 30ml provided was dissolved in 30ml of TBS (buffer pH 7.5 prepared previously for dot blotting). Thirty milliliters (30ml) of the blocking solution was poured into a 15 cm diameter petri-dish. The membrane was slowly slid into the solution avoiding formation of bubbles and then incubated for one hour. Antibody solution was prepared by dissolving buffer provided in 30ml of TBS and while agitating, 100µl of lgG-Rs was added in an Eppendorf tube. The blocking solution was discarded and antibody solution (30ml) added to the petri-dish. The petri-dish was covered with a lid to avoid evaporation and incubated for 2hr with gentle agitation. Two hundred and fifty microliters (250µl) of Tween-20 was mixed with 500ml of TBS. Antibody solution was discarded and unbound Rs-antibodies removed by washing the membrane in 30ml of TBS by agitating at 100 rpm 3 times for 3 minutes. The last washing was done by dissolving 30ml of conjugate buffer with 30ml of TBS while agitating and 100µl of conjugate goat anti rabbit antibodies was added. Finally, during the last washing, buffer was discarded and conjugate solution (30ml) added and incubated for 1hr while gently agitating.

To achieve colour development, the content of substrate buffer provided was dissolved in 100ml of distilled water and mixed thoroughly. The pH was adjusted by adding HCl solution up to pH 9.6 to end up with substrate buffer. Nitro blue Tetrazolium (NBT) / (BCIP), Toludine salt of 5-bromo, 4 chloro, 3 indolyl solution was prepared by dissolving the content of NBT in 800µl of solvent solution (DMF/NBT) and vortexed until completely dissolved. Then content of BCIP was dissolved by adding 800 µl of solvent solution (DMF/BCIP) mixing with a vortex until completely dissolved.

Conjugate solution was discarded and unbound conjugate GAR-lgG was removed by washing membrane with 30ml of T-TBS three times for three (3) minutes at the same time mixing using a vortex. One hundred microliter (100 µl) of NBT solution was added to a flask containing 25ml of substrate buffer drop by drop while agitating. Then, 100µl of BCIP solution was added still agitating the mixture. The last washing buffer was discarded and colour development solution was added (25ml per membrane). The reaction of purple colour development was checked after 20 minutes and then stopped by discarding the substrate solution and rinsing thoroughly the membrane with tap water and drying it with filter paper. The enzymatic reaction giving rise to a purple colouration in positive samples was checked with positive control.

5.3.2. Screening seed potato for viruses (DAS-ELISA kit CIP)

The detection of plant viruses by serological techniques was based on the reaction of specific antibodies with antigens (virus particles) in *vitro*. DAS-ELISA, was used in screening for the viruses in both local and imported potato varieties. The technique includes buffer preparation, coating, sample preparation, adding conjugate and colour development. Six types of buffers were

prepared namely coating buffer, Phosphate buffer saline (PBS), washing buffer (PBS-Tween: PBS-T), extraction buffer, conjugate buffer and substrate buffer. Antibody of the virus tested was detected and mixed well with coating buffer in each plate. This was repeated for each of the viruses tested. One hundred micro-liters (100µl) of antibody and coating buffer solution (coating solution) was added to each well in the plate avoiding spilling the content of the well. Using a micropipette, a new tip was used for each virus coating solution. The plate was placed inside a sealed polythene bag (4x6x6). This was incubated at 37^0C for 4 hours. During incubation, samples of 0.5g (2 to 3 small leaflets) were selected and extraction of 2.0 ml of buffer added and sample ground until homogenized. The plate was emptied, drained on absorbent paper towel and using pipettes, wells of the plate were filled with PBS-T (washing step). Conjugate was added to 100µl of the ground sample in the wells. Two wells of positive and healthy controls were prepared and filled with extraction buffer. Plates were sealed and incubated in the refrigerator at 4^0C overnight. Thirty five milliliters (35ml) of conjugate antiserum (IgG-AP) was mixed with 10ml of conjugate buffer and plates washed carefully avoiding content of one well spilling into the other. Ninety microliters (90µl) of conjugate solution was added to each well of the plate and incubated at 37^0C for 4 hrs. Substrate tablet was dissolved in substrate buffer to obtain substrate solution and 80µl of substrate solution was added to each well of the plate and the plates were left for 30 to 60 minutes at room temperature for the reaction to occur. Colour development was monitored and captured using ELISA reader.

5.4 Results

5.4.1 Detection of *Ralstonia solanacearum* from certified potato seed production system

The positive control turned purple but this was not observed in the tested samples indicating that there was no reaction even after 20 minutes. All varieties were negative for *R. solanacearum* indicating that the seeds were free from the disease (Table 5.1). Bacterial wilt was prominent in

the farmers' production system which was indicated by sudden wilting of the crop and oozing detected from tubers. Confirmation of the disease in the field was by use of quick diagnostic kit for detection of *Ralstonia solanacearum*.

Table 5.1: Results of serological test on detection of *Ralstonia solanacearum* from certified potato seed production system and registered seed merchants

Production site	Variety	Sample code	*Ralstonia solanacearum*
KALRO Tigoni	Desiree	QS014/004859	-Ve
	Asante	QS014/004860	-Ve
	Shangi	QS014/004861	-Ve
	Tigoni	QS014/004862	-Ve
	Kenya Karibu	QS014/004863	-Ve
	Kenya Mpya	QS014/004864	-Ve
	Kenya Mpya	QS014/004865	-Ve
	K. Mavuno	QS014/004866	-Ve
	Purple Gold	QS014/004867	-Ve
	Dutch Robjin	QS014/004868	-Ve
KALRO-Njabi-ni	Asante	QS014/003789	-Ve
	Tigoni	QS014/003790	-Ve
Kisima Farm Meru	Kenya Mpya	QS014/001254	-Ve
	Asante	QS014/001255	-Ve
(Registered Seed	Asante	QS014/001256	-Ve
	Asante	QS014/001257	-Ve
merchants	Sherekea	QS014/001258	-Ve
	Kenya Mpya	QS014/001259	-Ve
	Purple Gold	QS014/001260	- Ve
	Kenya Mpya	QS014/001261	-Ve
	Asante	QS014/001262	-Ve
	Asante	QS014/001263	-Ve
	Asante	QS014/001264	-Ve

5.4.2 Screening of certified potato seed for viruses using serological technique

All the potato seeds were free from the viruses tested (Table 5.2).

Table: 5.2 Screening of certified potato seed for viruses using serological technique

Production site	Variety	Sample Code	PVA	PVM	PVS	PVX	PLRV	PVY
KARLO-Tigoni	Shangi	SV-150210	-ve	-ve	-ve	-ve	-ve	-ve
	Asante	SV-150211	-ve	-ve	-ve	-ve	-ve	-ve
	Shangi	SV-150212	-ve	-ve	-ve	-ve	-ve	-ve
	Shangi	SV-150213	-ve	-ve	-ve	-ve	-ve	-ve
	Tigoni	SV-150214	-ve	-ve	-ve	-ve	-ve	-ve
	Sherekea	SV-150215	-ve	-ve	-ve	-ve	-ve	-ve
Kisima farm	Kenya Mpya	QS014/4019	-ve	-ve	-ve	-ve	-ve	-ve
	Tigoni	QS014/4020	-ve	-ve	-ve	-ve	-ve	-ve
	Asante	QS014/4021	-ve	-ve	-ve	-ve	-ve	-ve
	Sherekea	QS014/4022	-ve	-ve	-ve	-ve	-ve	-ve
	Desiree	QS014/4024	-ve	-ve	-ve	-ve	-ve	-ve
	Dutch Robin	QS014/4025	-ve	-ve	-ve	-ve	-ve	-ve
KALRO-Njabi-ini	Asante	QS014/4026	-ve	-ve	-ve	-ve	-ve	-ve
	Tigoni	QS014/40	-ve	-ve	-ve	-ve	-ve	-ve
Positive control			+Ve	+Ve	+Ve	+Ve	+Ve	+Ve

Key: PVA potato virus A, PVM potato virus M, PVS potato virus S, PVX potato virus X, PLRV potato leaf roll virus, PVY potato virus Y.

5.4.3 Detection of *Ralstonia solanacearum* from farmers 'own potato seed using quick diagnostic kit

All potato samples from the farmers' fields were positive for *Ralstonia solanacearum* (Table 5.3).

Table 5.3: Detection of *Ralstonia solanacearum* from farmers 'own potato seed using quick diagnostic kit

County	Variety	Code No.	*R. solanacearum*
Meru-Marimba	Shangi A	QS014/5448	+ve
	Sherekea	QS014/5449	+ve
	Asante	QS014/5450	+ve
	Tigoni	QS014/5451	+ve
	Kenya Mpya	QS014/5452	+ve
Nakuru Ndimu	Shangi B	QS14/5446	+ve
	Nyayo A	QS14/5445	+ve
Kiambu- Limuru	Merumugaruro	QS14/5454	+ve
	Tigoni	QS14/5455	+ve
Positive control	Sample no. 2	From the farm	+Ve

5.4. 4 Screening for viruses from farmers' own potato seed

All samples that were analyzed from the farmer's production system had PVX, PLRV and PVY viruses (Table 5.4). This correlates with the survey findings which showed that potato leaf roll virus was recorded in all counties surveyed and the percentage of prevalence was between 36.4% and 90.9%. Nakuru had the least percentage of 36.4% followed by Kiambu County with 40% while Nyandarua had the highest (90.9%). These results were confirmed using quick diagnostic kits for the specific viruses.

Table 5.4: Screening of farmers' potato seed for viruses using serological technique

County	Variety	Code number	PVA	PVM	PVS	PVX	PLRV	PVY
Meru-Marimba	Shangi A	QS014/5448	-ve	-ve	-ve	+ve	+ve	+ve
	Sherekea,	QS014/5449	-ve	-ve	-ve	+ve	+ve	+ve
	Asante	QS014/5450	-ve	-ve	-ve	+ve	+ve	+ve
	Tigoni,	QS014/5451	-ve	-ve	-ve	+ve	+ve	+ve
	Kenya Mpya	QS014/5452	-ve	-ve	-ve	+ve	+ve	+ve
Nakuru – Ndimu	Shangi B	QS014/5446	-ve	-ve	-ve	+ve	+ve	+ve
	Nyayo A	QS014/5445	-ve	-ve	-ve	+ve	+ve	+ve
Kiambu-Limuru	Merumugaruro	QS014/5454	-ve	-ve	-ve	+ve	+ve	+ve
	Tigoni	QS014/5455	-ve	-ve	-ve	+ve	+ve	+ve
Positive control			+Ve	+Ve	+Ve	+Ve	+Ve	+Ve

Key: PVA potato virus A, PVM potato virus M, PVS potato virus S, PVX potato virus X, PLRV potato leaf roll virus, PVY potato virus Y.

5.4.5 Detection of *Ralstonia solanacearum* from imported potato seeds

All the eighteen imported potato varieties screened were free from *Ralstonia solanacearum* that causes bacterial wilt in potato **(**Table 5.5).

Table 5.5: Detection of *Ralstonia solanacearum* from imported potato seed

Variety	Sample Code	Lab. ID	Lot No.	*R. solanacearum*
Soprano	QS014/5066	BACT/114/014	50397	-ve
Challenger	QS014/5067	BACT/115/014	11074	-ve
Taurus	QS014/5068	BACT/116/014	51788	-ve
Voyager	QS014/5069	BACT/117/014	50469	-ve
Lady Claire	QS014/5070	BACT/118/014	50397	-ve
Panamera	QS014/5071	BACT/119/014	11198	-ve
L. Olympia	QS014/5072	BACT/120/014	50397	-ve
Milva	QS014/5073	BACT/121/014	12387	-ve
L. Amarilla	QS014/5074	BACT/122/014	51787	-ve
Lady Rosetta	QS014/5075	BACT/123/014	52679	-ve
Sifra	QS014/5076	BACT/124/014	51340	-ve
Evora	QS014/5077	BACT/125/014	12086	-ve
Rodeo	QS014/5078	BACT/126/014	51660	-ve
Zafira	QS014/5079	BACT/127/014	12146	-ve
Carolus	QS014/5083	BACT/131/014	-	-ve
Manitou	QS014/5085	BACT/133/014	-	-ve
Saviola	QS013/004107	BACT/051/014	14-7306-1	-ve
Arnova	QS013/004108	BACT/052/014	-	-ve
Control	Sample no. 3			+Ve

5.4.6 Screening of imported potato seed for viruses using serological technique

The results show that all the seed tubers of the eighteen imported varieties screened were free from the viruses tested (Table 5.6). These results were confirmed using quick diagnostic kits for the specific viruses.

Table 5.6: Screening of imported potato seeds for viruses using serological technique

Variety	Sample code	PYDV	TSWV	TRV	PVY	TYLCV	PLRV
Soprano	QS014/5066	-ve	-ve	-ve	-ve	-ve	-ve
Challenger	QS014/5067	-ve	-ve	-ve	-ve	-ve	-ve
Taurus	QS014/5068	-ve	-ve	-ve	-ve	-ve	-ve
Voyager	QS014/5069	-ve	-ve	-ve	-ve	-ve	-ve
Lady Claire	QS014/5070	-ve	-ve	-ve	-ve	-ve	-ve
Panamera	QS014/5071	-ve	-ve	-ve	-ve	-ve	-ve
Lady Olympia	QS014/5072	-ve	-ve	-ve	-ve	-ve	-ve
Milva	QS014/5073	-ve	-ve	-ve	-ve	-ve	-ve
Lady Amarilla	QS014/5074	-ve	-ve	-ve	-ve	-ve	-ve
Lady Rosetta	QS014/5075	-ve	-ve	-ve	-ve	-ve	-ve
Sifra	QS014/5076	-ve	-ve	-ve	-ve	-ve	-ve
Evora	QS014/5077	-ve	-ve	-ve	-ve	-ve	-ve
Rodeo	QS014/5078	-ve	-ve	-ve	-ve	-ve	-ve
Carolus	QS014/5083	-ve	-ve	-ve	-ve	-ve	-ve
Zafira	QS014/5079	-ve	-ve	-ve	-ve	-ve	-ve
Manitou	QS014/5085	-ve	-ve	-ve	-ve	-ve	-ve
Arnova	QS013/3378	-ve	-ve	-ve	-ve	-ve	-ve
Saviola	QS013/3379	-ve	-ve	-ve	-ve	-ve	-ve
Positive controls		+Ve	+Ve	+Ve	+Ve	+Ve	+Ve

Key: PYDV potato yellow dwarf virus, TSWV tomato spotted wilt virus, TRV tobacco rattle virus, PVY potato virus Y, TYLCV tomato yellow leaf curl virus, PLRV potato leaf roll virus

Seed potato production from certified seed system of KALRO and registered seed merchants were free from *R. solanacearum* and viruses implying production sites were disease free. Seed potato production from informal seed sector were contaminated with *R. solanacearum* and viruses.

5.5 Discussion

Analysis of *Ralstonia solanacearum* and viruses was conducted at KEPHIS laboratory Muguga. The findings indicated that seeds from KALRO (Njabi-ini and Tigoni), Kisima and Turi farms (registered potato seed merchants) were free from *Ralstonia solanacearum* and viruses and this implied that the production sites were free from these diseases. This also indicated that seed originated from mini tubers produced through either tissue culture technique or aeroponics production systems do not carry these diseases. Previous research revealed that KALRO produce mini potato seed through aeroponics and tissue culture techniques. These mini potato seeds are multiplied in areas that are disease free and then supplied to other organizations and farmers (Muthoni *et al.,* 2010; Mbiyu *et al.,* 2012).

Imported potato seed were clean from bacterial wilt and viral diseases and this indicates that the mother seed is free and that they are produced in disease free areas. This fulfilled the phytosanitary conditions set by the Kenya Government on import of potato seed into this country to facilitate farmers in potato production (MOA, 2005; KEPHIS, 2011).

Potato seed from farmers' production system in Meru – Mariba, Nakuru – Ndimu and Kiambu – Limuru screened for bacterial and viral diseases were contaminated with *Ralstonia solanacearum* and virus PVX, PLRV and PVY. This implies that farmers obtained potato seed from diseased seed sources an indicator of farmers' seed production system being contaminated by bacterial wilt and viruses. It also indicates farmers' farms are not disease free. Most farmers sourced potato seed from their own farms, markets and from neighbours already contaminated and this contaminates the farms with *R. solanacearum* (Ateka *et al.,* 2001; Kinyua., 2014). Other studies indicate that bacterial wilt is widely spread in Kenya and 77% of potato farms are affected by the disease (Muthoni *et al.,* 2013).

The renewal of seed from reliable sources, namely KALRO by farmers, is limited. In this case, farmers reuse seeds from their own farms which are already contaminated by viruses. This causes an increase in virus build up in the production system. Previous research showed that viruses infect developing tubers and infection is carried over from season to season in "seed" tubers (Kabira *et al.*, 2006, Gallenberg *et al*., 2007).

Other countries such as South Africa provide for prohibition relating to occurrence of certain pathogens like bacterial wilt. The user of the land where bacterial wilt has been detected/ suspected to occur is expected to remove any plant or plant product infected, or suspected to be infected, disease occurs or is suspected to occur. In areas where bacterial wilt is suspected to be present, the area is placed under quarantine (Department of Agriculture, 1993).

European plant protection organization (EPPO) recommends inspection in the field and the testing of plant viruses (PVX, PVY, PLRV) using serology tests to confirm the absence of the virus. EPPO quarantine regulations emphasize on rejection of all seed infected with viruses when detected through serological tests (EPPO, 1996).

CHAPTER SIX

6.0 General discussion, conclusions and recommendations

6.1 General discussion

The study showed that bacterial wilt was distributed in all the five counties surveyed and was more prevalent in the high altitude areas of Nyandarua, Meru and Nyeri. This is due to lack of certified seed which forces farmers to obtain poor quality seeds from their own farms and neighbours and this contribute to disease spread. Previous research indicates that 77% of potato farms in Kenya are affected by bacterial wilt (Muthoni *et al.,* 2013). Research done in the past also had reported that altitude contributes to bacterial wilt disease incidence and there is reduction in the disease with increase in altitudes due to the very cold temperatures (Mwaniki *et al.,* 2016) but, this was not the case in this study because Nyandarua and Meru which are in the highlands had bacteria wilt prevalence of 82% and Nakuru 64%. *R. solanacearum* that causes bacterial wilt is reported to survive in soil temperatures as low as 4^0C and is considered to be a pathogen of warmer areas (Sullivan *et al.*, 2013). Bacterial wilt presence at altitude 2942 masl has been reported and this is attributed to factors, such as seed source (Ateka, *et al.,* 2001). This survey revealed that Meru County was growing most potato among the counties visited. The average farm size under potato production was raged from 0.310 to 0.655 ha for the counties and this limited long crop rotation. Small farm sizes limit long crop rotation and renewal of seeds resulting in increase in seed-borne disease incidence in potato growing regions (Lutaladio *et al.,* 1995). Poor quality seed planted for several years also show tuber degeneration due to virus and increase in incidence and prevalence of BW and PLRV in potato production regions (FAO, 2010).

Seeds from KALRO and registered potato seed merchants were free from *R. solanacearum* and viruses implying that seed are produced from disease free areas. Research reveals that KALRO produce mini potato seed through aeroponics and tissue culture techniques and are multiplied in disease free areas (Mbiyu *et al.,* 2012). Imported potato seed were free from bacterial wilt and viral diseases indicating that they were produced in disease free sites which fulfills phytosanitary requirements on import of seed potato (KEPHIS, 2011). Farmers' seed were contaminated with *R. solanacearum* and viruses implying that these farms were not disease free. Farmers source seed from neighbours and this contaminates the farms with *R. solanacearum* (Kinyua, 2014).

The study demonstrated that potato cultivars differ in the level of late blight resistance for both local and imported varieties at the three sites. The level of resistance also varied with increase in (height) altitude. Dutch varieties, Carolus and Arnova had AUDPC value as low as 1.0 in all sites in the field indicating that they have high resistance for the disease under Kenyan conditions. These varieties were comparable with local varieties Sherekea and Kenya Mpya which also had AUDPC values as low as 1, indicating the similar level of resistance to late blight. In the past, several potato varieties were tested for resistance to late blight and released to farmers. Since 1998, several varieties with late blight resistance have been released in Kenya (Lung'aho *et al.,* 2006). However, Sherekea and Kenya Mpya seem to have high level of resistance to late blight. Sherekea is also resistant to viruses PVY and PLRV while Kenya Mpya is resistant to PVY and PVX (Onditi *et al.,* 2012). The study showed that Kenya Mpya and Sherekea were resistant to late blight in all the sites with high rainfall and low temperature of 18^0C and below and in warmer area with low rainfall and average temperatures of 22^0C and above. Other local varieties Tigoni, Kenya Mpya, Nyayo, Asante, Shangi and Sherekea had very low AUDPC values under greenhouse conditions. Previous research reported low AUDPC

values in such varieties and this indicated the possession of minor resistance genes *P. infestans* and potato interaction (Cañizares and Fobes 1995; Lee *et al.*, 2001).

The eighteen imported varieties including Soprano, Lady Olympia, Lady Claire, Lady Rosetta, Panamera, Sifra, Evora, Taurus, Milva, Lady Amarilla, Rodeo, Voyager, Challenger, Carolus, Arnova, Saviola, Manitou, and Zafira, had high AUDPC values of between 500 and 2250. This shows that imported germplasms are very susceptible to the late blight disease under greenhouse conditions and to an expert, field conditions with exception of Carolus and Arnova. Disease severity was ranging from susceptible potato varieties to resistant ones with imported varieties having AUDPC of between 500 and 2250 and local as low as 10.

Diseases, pests and other parameters affect potato yields and therefore, depending on individual variety resistance to disease, yields of various potato cultivars are also different. The spread rate of late blight disease depends on rainfall and temperature in the growing regions. Sherekea variety produces mean yields of between 45 to 50 t/ha and Kenya Mpya 40 to 45 t/ha (Onditi *et al.,* 2012). There was varietal difference in yield for the potatoes tested. Arnova variety had highest mean yield of 18.6 t/ha while Carolus variety had highest mean yields of 19.5 t/ha in areas with low rainfall and temperatures of between 20^0C and 22^0C indicating adaptability to this climatic conditions. The study shows that the performance of these two imported varieties Arnova and Carolus was higher than other local and Dutch varieties except Sherekea and Kenya Mpya at various sites. Sherekea, Kenya Mpya and Shangi are high yielding and resist late blight differently depending on the climatic condition of different areas. Arnova and Carolus could be compared with Kenya Mpya and Sherekea in terms of high yields and resistance to late blight disease at different field conditions.

6.2 Conclusion

There are two seed production systems in Kenya namely formal and informal systems later being practiced by most of the farmers. Certified seed system has l prevalence for bacterial wilt and viruses while the prevalence is high in farmers' seed system. Farms for majority of the farmers are small and farmers practice short crop rotation cycles. Most farmers obtain seed potato from their farms, market and neighbors do not renew seed potato from reliable sources.

The levels of infection vary in the various production systems. Imported varieties did not have bacterial wilt and viral diseases. The local certified seed also did not have bacterial wilt and viral infection but, farmers produced seed had both diseases.

Cultivars tested both local and imported, showed varied levels of late blight resistance in the field. Local varieties led by Sherekea and Kenya Mpya showed higher resistance to late blight than all the other local varieties. All the imported varieties had low resistance to late blight except for Carolus and Arnova which showed high resistance to late blight under field conditions.

Carolus and Arnova were found to be adaptable to local climatic conditions and had high yields while other imported varieties yielded below average. Carolus and Arnova can compare with Sherekea and Kenya Mpya in terms of resistance to late blight disease.

6.3 Recommendations

- There is need for clean seed to satisfy farmers demand hence more farmers should be contracted by the M OA to produce more clean seed potato in post control programmes.

- KALRO and seed merchant in the country should increase capacity in production of certified seed potato to meet farmers' demand for clean seed potato.

- Farmers should avoid using seed potato from their own farms, neighbours and market but should always obtain clean seed potato from certified seed production sources to avoid introduction of these diseases to their fields.

- Three years' rotation with rhodes grass, maize, beans, cabbages and other unrelated crops may be recommended for testing as a mean of reducing bacterial wilt and viruses.

- Ecological adaptation of Carolus and Arnova varieties should be done with a view of releasing these varities to farmers in Kenya.

- Locally bred varieties Kenya Mpya and Sherekea can be recommended for more seed multiplication using clean starter seed potato because of their high yields and resistance to late blight.

- Kenya Mpya, Sherekea, Carolus and Arnova can be recommended for use in the existing breeding programmes as donors for late blight resistance genes in breeding for resistance to late blight.

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APPENDICES

Appendix 1: Questionnaire

Questionnaire on potato production systems and farmers' practices survey in Meru, Nyeri, Nyandarua, Nakuru and Kiambu counties

GENERAL INFORMATION

Introduction

Name of data collector……Questionnaire No……Date…….

Name of farmer (optional)……

Respondent owner/employee/manager……

Location and Geographical Positioning System

County……Locality……

Altitude……. ……Agro Ecological zone……

Elevation……. South…….

East……

Potato production system/farmers practices

Farm size under potato production……

Purpose of farming – seed / ware……

Seed source…….

Seed renewal……

Other crops grown in the farm……

Cropping cycle (crop rotation) ……

Bacterial presence/absence in the farm……

Potato leaf roll presence/absence……

Appendix 2: Meteorological data

Metological data summary from UON, Gene Bank Mguga and Njabi-ini KALRO

County	Average temperature	Average rainfall
Nyandarua – Njabi	13^0C	2,300 mm
Kikuyu - Muguga – (GRRI	18^0C	1,900 mm
Kikuyu – UON – Kabete	22^0C	1,250 mm

Appendix 3.1: Disease diagnosis using Pocket Diagnostic Kit (Quick Diagnostic Kit)

The process of using pocket diagnostic kit for the field diagnosis of diseases (*Ralstonia solanacearum* and Potato viruses) is described below.

1. A sample was selected and cut into small pieces
2. Then put into a bottle containing buffer and ball bearings
3. It was shaken for 30-60 seconds to break up the sample then the liquid was allowed to settle
4. The liquid was drawn into the pipette avoiding sample debris and air bubbles
5. Keeping the test device level, 2 drops of the extract were added into the sample well of the device
6. Valid results were observed within 10 minutes

Test interpretation

One-line present only at the C line indicated a negative result.

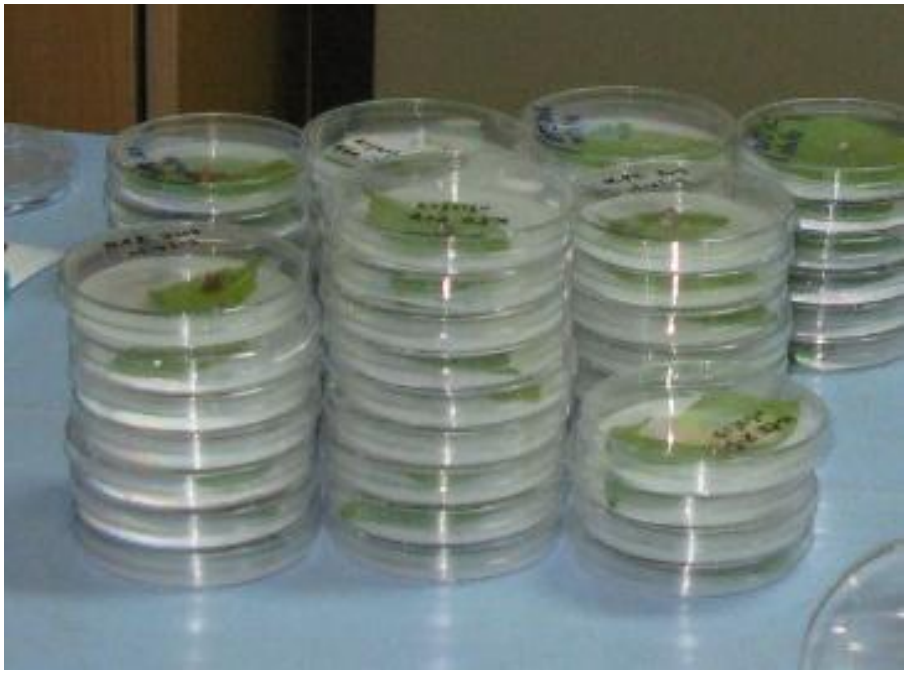
Two lines present, at both the T and C line, indicated a positive result.

The absence of a line at C indicated the test had failed and must be repeated

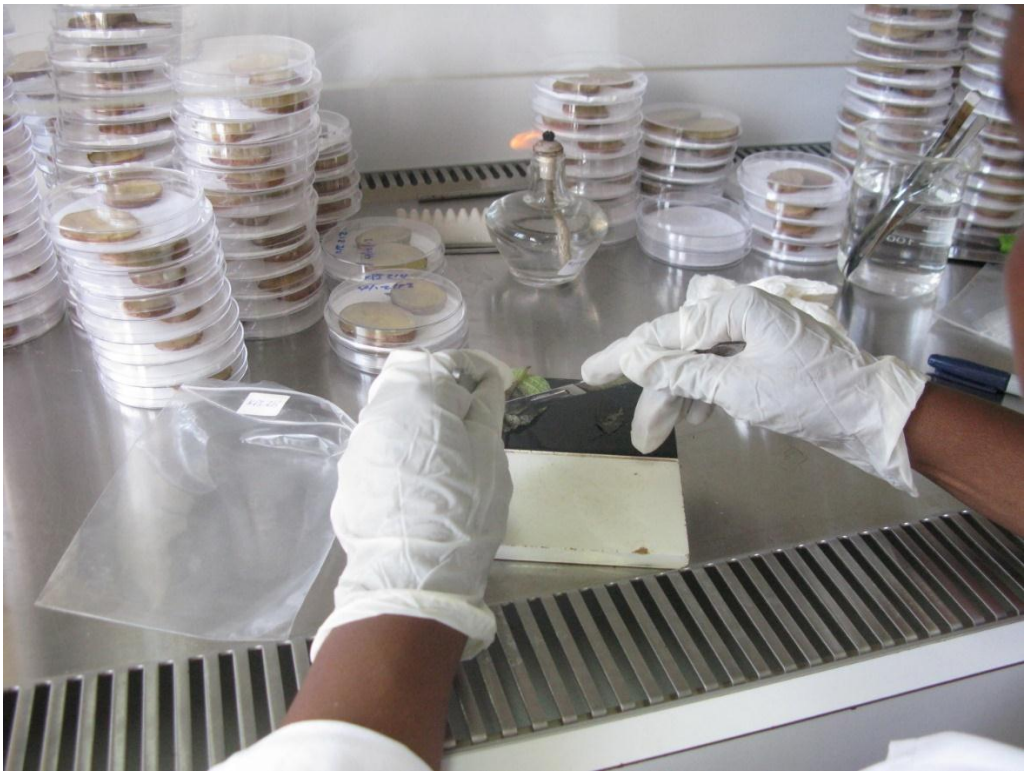
Appendix 3.2: Picture of pocket diagnostic Kit (Quick diagnostic kit)

Appendix 4.1: Symptoms of late blight on potato leaves caused by *P, infestans*

Appendix 4.2: Surface sterilization of potato tubers using 70% alcohol.



Appendix 4.3: Young leaves infected by *P. infestans* used in the inoculation of potato tubers



Appendix 4.4: Preparing leaves infected by *P. infestans* for inoculating potato tubers.

Appendix 4.5: Inoculating potato tuber with leaf infected by *P. infestans* to grow cultures of the pathogen

Appendix 4.6: Preparation of inoculum from cultures of *Phytophthora infestans*

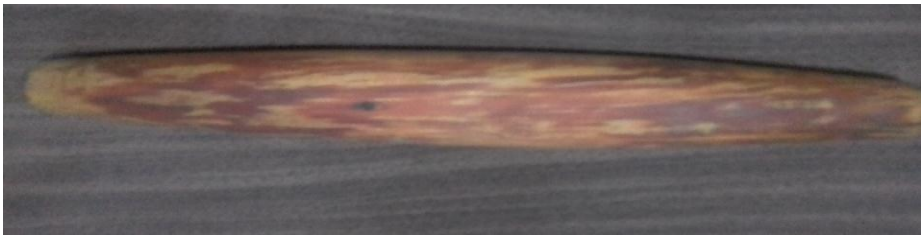
Appendix 4.7: Potato plants before infection of *P. infestans* at the University of Nairobi

Appendix 4.8: Potato plants after infection of *P. infestans* at the University of Nairobi

Appendix 4.9: Potato plants before infection of *P. infestans* at the Gene Bank site

Appendix 4.10: Potato plants after infection of *P. infestans* at the Gene Bank site

Appendix 4.11: Potato plants after infection of *P. infestans* at Njabi-ini site



Appendix 5.1: Rolling pins