STATUS OF POTATO BACTERIAL WILT IN NAKURU COUNTY (KENYA) AND ITS MANAGEMENT THROUGH CROP ROTATION AND SOIL AMENDMENTS

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A thesis submitted to the Graduate School in partial fulfilment for the requirements of the Doctor of Philosophy Degree in Plant Pathology of Egerton University

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DECLARATION AND RECOMMENDATION

Declaration

I declare that this thesis is my original work and has not been presented to any University for any degree or any other award.

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Recommendation

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ABSTRACT

Bacterial wilt caused by *Ralstonia solanacearum,* is the second most damaging potato disease in tropical and sub-tropical regions causing up to 75% of crop loss and affects 77% of potato farmers in at least 10 Counties in Kenya. Lack of actual information on the distribution of bacterial wilt limits application of strategic approaches in curbing the disease in Nakuru county and other parts of Kenya. A survey was undertaken using a semi structured questionnaire and personal observation to collect data in nine Wards to establish it's occurrence in Nakuru County. The results showed that the average yield of potato in the nine Wards surveyed was 14.5 ton/ha. Bacterial wilt prevalence in the County varied from 100% in Bahati Ward, to 35.7% in Mauche Ward. The wilt incidence in the farms varied from 0 to 41%. A biochemical analysis of *R. solanacearum* isolates collected from bacterial wilt infected potatoes and common weeds within the farms indicated that biovar 2 and 3 were existent in the County. Seed source, potato variety and lack of seed renewal were the main contributing factors to bacterial wilt in the County. Consequently, crop rotation and soil amendment experiments were laid out in RCBD design in plots of 3m x 3m in Egerton University, Njoro and KALRO, Kabete to evaluate their effect on bacterial wilt. The results indicated that pre-cropping potato with spring onion and barley resulted to significantly lower wilting incidence in potato with a grand mean of 8.3% across the two locations. Potato-*Dolichos lablab*-Potato and Cabbage-*Dolichos lablab*- potato had the highest potato yield of 19.9 tons/ha and 19.7 tons/ha respectively. In the long season crops experiment, potato planted after *Desmodium intortum* recorded the lowest mean wilt incidence (18.7%) and highest mean yield (16.2 tons/ha). The soil amendment results showed that NPK + Black majik is a promising combination of organic and inorganic fertilizer which can be used to increase yields in the short term and also improve reduce bacterial wilt incidence. Black majik + NPK, cow manure, Takataka compost and neemgold significantly reduced the wilt incidence at Egerton site compared to the negative control (no amendment) $F(7,128) = 2.830$, $P<0.05$. Sourcing for clean seed from approved seed potato dealers should be encouraged among farmers. Rotations involving spring onion with the locally grown cereals can be utilized in curbing bacterial wilt. Reinforcing inorganic fertilizers with organic amendments is essential in the short term realization of high yields and improving soil health. This study provides comprehensive information on the status of bacterial wilt in Nakuru County and also offers affordable and applicable measures to farmers in curbing bacterial wilt.

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LIST OF ABBREVIATIONS AND ACRONYMS

ADC	Agricultural Development Co-operation
AMMI	Additive main effects and Multiplicative interaction
ANOVA	Analysis of Variance
BBCH	Biologische Bundensanstalt Bundessortenant und Chemische Industrie
CFU(s)	Colony Forming Units
CIP	International Potato Center
CPG	Casamino acids, Peptone and Glucose medium
DNA	Deoxy-ribonucleic Acid
DIMBOA	2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3(4H)-one
EDTA	Ethylene Diamine Tetra Acetic acid
FAO	Food and Agricultural Organization
FYM	Farm Yard Manure
GPS	Geographical Positioning System
GSL	Glucosinolates
HCDA	Horticultural Crops Development Authority
ITC	Isothiocyanates
KALRO	Kenya Agricultural and Livestock Research Organization
MoA	Ministry of Agriculture
NCM-ELISA	Nitro Cellulose Membrane- Enzyme Linked Immunosorbent Assay
NGO	Non-Governmental Organization
PCA	Principal Component Analysis
R3Bv2	Race 3 Biovar 2
RCBD	Randomized Complete Block Design
SMSA	Semi Selective Medium Agar
SPSS	Statistical Package for the Social Sciences
TN	Total Nitrogen
Tukey's HSD	Tukey's Honestly Significant Difference
TZC	Tetrazolium Chloride
TOC	Total Organic Carbon
WI WAP	Wilting Incidence Weeks after Planting

CHAPTER ONE

INTRODUCTION

1.1 Background information

Potato (*Solanum tuberosum* L.) is the world's fourth important food crop after maize, wheat and rice (FAO, 2008). World potato production reached a record 320 million tonnes in 2007 and production in the developing countries has almost doubled since 1991, with a corresponding increase in consumption. Kenya is the fifth largest potato producer in Sub- Saharan Africa, with an output of 790,000 tonnes in 2006 (FAO, 2009). The crop is the second most important staple food crop after maize in Kenya (MoA 2010) and plays a major role in national food and nutritional security. Potato in Kenya is grown on about 108,000 ha with an annual production of over 2.5 million tonnes in two growing seasons (MoA, 2010). In Kenya, potatoes are mainly cultivated in the high altitude areas such as the slopes of Mt. Kenya, parts of Laikipia, on both sides of the Aberdare ranges, along Mau Escarpment, Nandi Escarpment and Cherangani hills (MoA, 2010). Bacterial wilt and low soil fertility are major factors that contribute to low potato yields in farmers' fields (Lemaga *et al.,* 2001).

Bacterial wilt caused by *Ralstonia solanacearum,* is the second most damaging potato disease in tropical and sub-tropical areas and is exacerbated under conditions of moisture stress, low soil fertility and low pH. *Ralstonia solanacearum* Race 3 biovar 2 (R3b2), formerly known as *Pseudomonas solanacearum* is one of the most damaging pathogens on potato worldwide (Janse, 1996). It has been reported to affect 3.75 million acres in approximately 80 countries with global damage estimates exceeding $950 million per year (Floyd, 2007). The disease occurs in all potato growing areas and can cause losses of up to 75% in crops. Bacterial wilt ranks fifth in priority in areas of potato improvement in developing countries (Schulte-Geldermann, 2013). Bacterial wilt is also considered as a top priority in improvement of potato sector in Sub-Saharan Africa (Fuglie, 2007). Kenyan potato industry is threatened by bacterial wilt because soils in most production areas are infested with the wilt-causing bacterium and over 50% yield losses have been reported (Ajanga, 1993). A survey undertaken in ten major potato growing Counties of Kenya showed that bacterial wilt was the most prevalent potato disease affecting 77% of potato farmers (Kaguongo *et al.,* 2013). Productivity of soils in the highlands of Kenya is limited

by low nitrogen, phosphorous and potassium. This forces most farmers to apply inorganic fertilizers, which in turn reduces the soil pH. Such soil environment has been reported to be conducive to bacterial wilt therefore supporting multiplication of the pathogen (Ramesh and Bandyopadhyay, 1993). Continuous cultivation also contributes to poor crop performance and increase nematodes infestation. Disease severity increases if *R. solanacearum* is found in association with root nematodes, which changes the physiology of the plants, thereby increasing its susceptibility to the disease (Akanni and Ojeniyi, 2008). Infected seed and soil are reported to be the main means of bacterial wilt spread and the main control method preferred by farmers in this area is uprooting of wilting plants (Kaguongo *et al.,* 2009).

Geographic distributions of pathogens are as a result of dynamic processes involving host availability, susceptibility, abundance and suitability of climatic conditions and farmers' practices. Information on the distribution of *Ralstonia solanacearum* in a given region relative to the host availability, climatic conditions, and farmers' practices among other factors is significant in strategic policy making. There are five races and five biovars of *R. solanacearum.* The most common strain is race 3, biovar 2 (R3b2), which dominantly attack the Solanaceae family (Champoiseau *et al.,* 2009) and particularly potato. The strain is reported to survive in cold environments (Messiha *et al.,* 2007) but is more severe in temperature ranges of 24°C - 35 °C with an optimum of 27°C. Biovars 2 and 3 are reported to be present in Kenya and therefore identifications of biovars will improve disease control such as development of resistant varieties and elimination of alternative hosts (Dhital *et al.,* 2001; Nyangeri, 2011).

High prevalence of pests and diseases is among the threats emphasized by the ministry (MoA, 2015). Potato production has not been sustainable with the current rotation systems because of unsuitable management of soil borne pests and diseases and a continuous deterioration of soil structure and fertility (Struik and Wiersema, 1999). One of the biggest problems facing the control of potato bacterial wilt is that farmers are not aware of the right crops to use in rotation in addition to small land sizes (FAO, 2013). The option of leaving the land fallow as a bacterial wilt control strategy is also restricted to very few farmers due to small parcels of land. Most farmers practice potato production in bacterial wilt infested soils, which necessitates appropriate crop rotation regimes. Crop rotation is one of the cultural strategies that have been used in inoculum reduction in control of diseases. The major emphasis in crop rotation is the length of rotation,

type of cropping sequences and incorporation of crop residues in the soil. Studies have shown that as the length of time between susceptible host increases, there is a decrease in the pathogen population (Hopkins *et al*., 2004). Incorporated vegetative materials of crops used in rotations are also reported to have an impact on the disease in the soil. They are reported to release volatile compounds into the soil which affect the pathogens or improve microbial activity thereby increasing soil organic matter. This increases microbial competition in the soil which leads to suppression of pathogens (Hopkins *et al*., 2004). Crop rotation is therefore a promising control measure to farmers in reduction of bacterial wilt if the right crops are identified for the rotations and favourable cropping sequences.

Soil fertility is known to be among the major yield limiting factors in potato production according to Gildemacher *et al*. (2009). Potato yields in Kenya have remained low with an average of 7.7 tonnes per hectare (Janssen *et al*., 2013). The gap between actual and potential yields in potato production is aggravated by insufficient nutrient supply to the soil and a large intake of nutrients by the high demanding potato crop (FAO, 2009). Application of nutrients is done through the use of inorganic and organic fertilizers. Proper fertilization in potatoes produces healthy plants that are able to tolerate pests and diseases. Organic matter increases soil friability, soil fertility and beneficial organisms which positively influence soil temperatures and moisture levels. Organic matters such as compost increases the diversity and quantity of microbes in the soil which result to suppression of pathogens (Nakhro and Dkhar, 2010). Organic matter in potato production is essential since there is release of nutrients in stages suiting plants ability to absorb them. The role of inorganic fertilizers cannot also be ruled out since they are a source of readily available nutrients (FAO, 2006). Incorporation of both organic and inorganic fertilizers stimulates microbial soil life and facilitates decomposition processes which improve the overall soil health. Use of soil amendments in potato production in bacterial wilt infested soils is important in control of soil borne pathogens, improved soil fertility and consequently increased yields (Lemaga *et al*., 2001; Hopkins and stark, 2003).

1.2 Statement of the problem

The potato industry in Kenya is threatened by bacterial wilt and over 50% yield losses have been reported. It affects more than 77% of potato farmers in the country. Low soil fertility and soil organic matter, poor physical and chemical properties associated with continuous cultivation of

potatoes often exacerbates the build-up of bacterial wilt pathogen. Low organic matter reduces microbial population and their diversity thereby reducing microbial competition against pathogens. This gives an advantage to *R. solanacearum* in the presence of a susceptible host. Poor soil fertility also reduces the vigour of the potato plants making them more susceptible to the disease and other pests such as nematodes that enhance infection. These factors culminate to reduced potato yields. This problem is found in most potato producing areas including Nakuru County. Although, research has shown bacterial wilt as an important disease in potatoes in Nakuru County and other parts of Kenya, little information is documented on its prevalence, incidence and farmers practices in these areas. Lack of relevant information limits use of strategic approaches in curbing the disease. Several management strategies have also been employed to curb the disease such as chemical control, breeding for resistance, cultivar resistance, and biological control among others. However, most of these control strategies are limited; e.g. quarantine is limited due to uncontrolled movement of seed across borders. Chemical control is not non-selective for target pathogen and therefore destroys other beneficial organisms. Use of biological control agents (BCAs) are also limited by inconsistent colonization, narrow host range, instability in effectivity among other factors. Utilization of cultivar resistance is also limited due to linkage with undesirable traits. Crop rotation is among the recommended cultural practices of which majority of farmers use, however, some of the crops used do not correspond to the varying eco zones, environmental characteristics and farm level circumstances. Most of the research done on crop rotation, organic and inorganic soil amendments mainly focuses on yield responses and nutrient recovery, while information is lacking on effective management strategies focussed on reduction of bacterial wilt and increased soil microbial activity. Lack of information on the effect of cultural practices on bacterial wilt may culminate in increased expenses in amending the soil with a focus on increasing potato yield while the losses due to bacterial wilt continue rising. The study addressed the above problem by establishing the prevalence and incidence of the disease in Nakuru county and evaluating the role of crop rotation and soil amendment in it's management.

1.3 Objectives

1.3.1 General objective

To contribute to improved potato production through management of bacterial wilt caused by *Ralstonia solanacearum* in Nakuru County using different management strategies.

1.3.2 Specific objectives

(i) To evaluate the status of potato bacterial wilt in the major potato producing Wards of Nakuru County.

(ii) To evaluate the effect of several crop rotation sequences on bacterial wilt of potatoes.

(iii) To evaluate the effect of organic and inorganic soil amendments on bacterial wilt and on yield of potatoes.

1.4 Null hypotheses

(i) The prevalence of potato bacterial wilt is not significantly different in the potato producing Wards of Nakuru County.

(ii) Different crop rotation regimes have no significant effect on bacterial wilt of potato.

(iii)Organic and inorganic soil amendments have no effect on bacterial wilt and potato yield.

1.5 Justification

To realize the full potential of potato production in Nakuru County, it is important to assess the actual situation in the region with regard to bacterial wilt status and contributing factors. One of the mandates of the Ministry of Agriculture (MOA) in Kenya in their strategic plan (2013-2017) is to control diseases and pests by employing activities such as development of control strategies for diseases and pests and developing strategies for surveillance and monitoring diseases. This study therefore contributes to this mandate by establishing the status of potato bacterial wilt in the County and offering substantial basis for the relevant stakeholders in combating the disease. Determination of *R. solanacearum* distribution and the existing biovars is valuable in identifying areas of high risk in order to improve site specific control of bacterial wilt in Nakuru County. With such information at hand, it is possible to develop relevant, affordable and feasible control

strategies. Bacterial wilt control through crop rotation, intercropping and organic manuring has been attempted and has not only been found to suppress the pathogen, but also improve the soil fertility. Since no single control method has been reported to be effective in managing bacterial wilt, evaluation of several control methods is an effective strategy that will reduce bacterial wilt in the soil. Effective crop rotation systems that utilize crops being grown by farmers and adapted to an area also provides farmers with alternative sources of food and income as they control the disease. Determination of effective organic and inorganic amendments obtained from internal farm resources and also readily available and affordable external resources is therefore significant in reducing bacterial wilt and at the same time increasing yields.

CHAPTER TWO

LITERATURE REVIEW

2.1 Economic importance of potato

Potato is the fourth most important food crop in the world and falls behind rice, maize and wheat. By the year 2006, the world production was at 315 million tonnes according to FAO (2008). In the year 2009, potato production was at 330 million tonnes with Africa contributing 18 million tonnes. The total area of production worldwide is reported to be on the decline with an observed increase in area of production in the developing countries. This is attributed to the varying agro ecological zones and adaptability of a greater number of varieties in the developing countries (Gastelo *et al.,* 2014).

Kenya was the fifth largest potato producer in Sub-Saharan Africa with an output of 790,000 tonnes in the year 2006 (FAO, 2009). Production in 2010 was estimated to be 1 million tonnes with at least 800000 potato growers (FAO, 2013). It is the second most important food crop after maize with cultivation on at least 108,000 hectares per year. Most of the potato farmers are small scale farmers (98%) and contribute to 83% of the national production (Janssen *et al.,* 2013). Nakuru County ranks second to Nyandarua County in total area under potato production in Kenya. According to 2011 and 2012 statistics, the total area under potato production was approximately 16,053 and 16,804 hectares respectively (HCDA, 2012; FAO, 2013; Kaguongo *et al.,* 2013). Potato is a strategic food commodity in boosting food security in Kenya. It is a crop of high nutrition value, rich in protein, calcium, potassium and vitamin C with a good amino acid balance. The nutritive value of potato is considered to be higher compared to cereals such as maize and wheat and legumes such as beans, soybean and peas when cooked and consumed with the skin. It is reported to produce twice as much protein per hectare compared to dry beans (FAO, 2013). Due to its high acceptability in many Kenyan households, it plays a major role in the health of the community. The possibility of usage of potato in crop diversification improves food security, mitigation of risk and also allows for conservation of biodiversity. It also encourages flexibility for producers in terms of evolving market conditions, horizontal and vertical diversification. It is an important income generating crop especially for small scale

farmers. It employs at least 800,000 farmers as producers in Kenya while an extra 2.5 million are engaged as market agents, transporters, processors, vendors and exporters (FAO, 2013).

2.2 Potato production characteristics

Potato is a cool weather crop which grows at altitudes of 1500-4200 m ASL in tropical areas. Optimum growth temperatures range between 18^{o}C to 20^{o}C with night temperatures of approximately 16^{o}C which facilitates tuberization. A soil pH of 5.0-5.5 is conducive for potato production with loose moist and well drained soils being recommended (FAO, 2009). Potato production in Kenya is done in the high altitude areas i.e. 1500-3000 metres above sea level (Janssen *et al.,* 2013). Potato has advantages over most food crops in that it allows for crop diversification, it is a highly productive crop, has a short growth cycle, adapts to a wide range of climates, it can be produced in the main growing seasons and off seasons, it is not very perishable, has high yield potential per unit area of production compared to most crops, it is easy to intercrop and rotate it with other crops (FAO, 2013).

Potato production in Kenya is done at small scale and accounts for at least 50% of the household acreage. Large scale potato farmers practice monoculture whereas small scale farmers diversify the number of crops in their farms (FAO, 2013). Small scale farmers also grow their potato crop without rotating with other crops; however some farmers practice rotation with crops such as maize wheat and barley. Most potato production is rain fed and is planted twice per year; in the long and short rains, however this varies with the geographical areas (FAO, 2013). Potato production has the potential of reaching 100 tonnes per hectare under irrigation (Potatoes South Africa, 2014/15). The average yield of potato in Kenya is reported to be 7.7 tonnes per hectare (Janssen *et al.,* 2013).

2.3 Constraints to potato production

Potato production is constrained by several technical, socio-economic, policy and institutional factors. Technical constraints cited to affect potato include soil and seed-borne insect pests and diseases, post-harvest constraints of fresh tubers, lack of efficient seed systems e.g. multiplication and distribution of certified seed tubers, inadequate resource allocations (FAO, 2009). Among the abiotic factors, low soil fertility caused by continuous cultivation without soil replenishment with the right nutrients has significantly affected potato yields (Muthoni and

Nyamongo, 2009). Continuous application of mineral fertilizers, application below the recommended rates and use of these fertilizers on acidic volcanic soils in Kenyan highlands are some of the causes that have aggravated low soil fertility (Janssen *et al.,* 2013).

Rain variation is reported to be a threat to potato production in regions of central Kenya such as Oljororok and Meru (Wang'ombe and Van Dijk, 2013; Karanja *et al.,* 2014). Seasonality of production based on the bimodal rainfall patterns limits profitability due to gluts and lean times. A study carried out in Guinea revealed that climate-related factors such as drought, wildfire and flooding were constraints influencing potato production (Tolno *et al.,* 2016). Drought is considered of importance since it influences productivity by affecting flowering, tuberization, canopy growth and tuber bulking (Gastelo *et al.,* 2014).

Other important factors limiting potato production in Kenya include social-economic constraints such as limitation in mechanization, challenges in marketing of produce due to poor infrastructure, seasonality in production and supply, exploitation by brokers, un-standardized packaging (Gildemacher *et al.,* 2009; Muthoni and Nyamongo, 2009; Janssen *et al.,* 2013; Kaguongo *et al.,* 2014). Lack of access to centralized market information by the farmers and poor linkages to market are challenges to potato marketing (Taiy *et al.,* 2016). Inaccessibility to agricultural credits limits the farmers' productivity which affects the quantity and quality of potatoes (Janssen *et al.,* 2013; Makoni *et al.,* 2014).

Lack of stringent government and institutional policies expose farmers to corrupted deals and poor linkage systems. Adulterated inputs were highlighted by Ahmad *et al.,* (2005) as another constraint in potato production. Non-compliance to legal and industry standards limit small scale farmers from international markets and other stringent wholesale outlets such as supermarkets. Despite the various acts of parliaments existing, strategy documents and legal notices that govern production, their implementation has not been fully realized. According to FAO, (2013), most of policies and strategies affecting potato production need to be reviewed and updated. Most small scale farmers are ignorant on the use of technology in accessing agricultural services such as consultations, market prices, electronic money transfers and supplying and demand using cell phones, internet and media (Makoni *et al.,* 2014). Failure by the governments and institutions mandated to work with farmers in offering information on the advancement of technology adversely affects the productivity and profitability.

Late blight is considered as the most important disease of potato worldwide (Forbes, 2008; Gastelo *et al.,* 2014). Biotic factors such as late blight, bacterial wilt and potato tuber moth are the major problems affecting potato farmers in Nakuru County. These are ranked as the most important constraints in most of the Wards of Nakuru County among other constraints such as marketing, poor seeds, lack of standard weight and measures (FAO, 2009).

2.4 Bacterial wilt as a constraint to potato production

Bacterial wilt is a soil borne disease caused by the bacterium *Ralstonia solanacearum* as described by Yabuuchi *et al*., 1995. Bacterial wilt is estimated to affect 1.75 million hectares of land in 80 countries according to Walker and Collion (1998) and affects more than 200 plant species. It is listed as a quarantine pathogen since it is very destructive and difficult to control (Guchi, 2015). As observed by Gildemacher *et al.* (2009), it is a major and frequent threat to potato production in Kenya, Uganda and Ethiopia. This survey which was carried out in the three countries, showed that 59% of all the sampled plots in the highlands were infested with bacterial wilt. The pathogen is able to survive in the soil for long periods of time, eight years or longer (Potatoes South Africa, 2015). It spreads through contaminated water ways and irrigation water. Alternative hosts such as weeds enable the pathogen to survive and multiply even in the absence of the primary host (Guchi, 2015).

Several management strategies are practiced to curb the disease. These include chemical control, cultural practices such as crop rotations and cultivar resistance, organic amendments, biological control, among others (Yuliar and Koki*,* 2015).

2.5 Characteristics of *Ralstonia solanacearum*

Ralstonia solanacearum is a gram-negative, rod-shaped, strictly aerobic bacterium that is 0.5-0.7 × 1.5-2.0 µm in size with a single polar flagellum. It has a twitching motility over solid surfaces such as media using a type IV pili system (Liu *et al*., 2001). The optimal growth temperature is between 28 and 32°C. In culture, individual bacterial colonies are usually visible after 36 to 48 hours growth at 28°C. Colonies of the normal or virulent type are purple coloured with a white or cream periphery, irregularly shaped, highly fluidal, and opaque (Champoiseau *et al.,* 2009). Classification of strains and species of any pathogen is important in any genetic improvement of a crop, understanding the diversity of any species and also appreciating plant-pathogen

interactions. Several characterization methods are used in identification of races, biovars, phylotypes and sequevars of *R. solanacearum* (Sullivan *et al.*, 2015). Several biochemical tests done in characterization consist of gram staining, potassium hydroxide test, catalyse oxidase test, levan production from sucrose, Kovacs oxidase test, production of fluorescent pigment, gas production from nitrate, lipase activity on Tween 80 agar, arginine dihydrolase reaction and oxidation and fermentation of glucose (Zubeda and Hamid, 2011). Kovacs oxidase test is used to classify strains based on their mode of respiration. Five biovars have been identified based on their ability to oxidize various disaccharides (cellobiose, lactose and maltose) and hexose alcohols (dulcitol, mannitol and sorbitol) expressed by change in the medium colour from green (neutral pH) to yellow (acidic pH) (Figure 3.1).

Pathogenicity tests are used to classify *R. solanacearum* isolates based on their infectivity to different hosts and therefore describes the different races. Avirulent strains have been identified in pathogenicity tests of which they are attributed to loss of virulence due to repeated subculture *in vitro* (Ibrahim *et al.,* 2005). Variations on the pathogenicity of strains from different hosts have been described by Rodrigues *et al.,* (2012). Race 1, biovar 1, 3, 4 is distributed in Asia, Africa, Australia, North and South America. It has a wide host range such as ginger, olives, chilli pepper, peanuts tobacco and other *Solanum* species. Race 2 biovar 1 is distributed in North and South America and has been isolated in *Musa* spp. and tomatoes, Race 3 biovar 2 is widely distributed worldwide and affects *Pelargonium* and most *Solanaceous* spp. Race 4, biovar 3 and 4 occurs in Australia, India and Asia and affects ginger (Sullivan *et al.,* 2015).

Further molecular analysis is applied in classification of phylotypes. These phylotypes are based on their ancestral relationships and geographical origins of the strains. A classification scheme has been described for strains of *R. solanacearum* to twenty three sequevars and four phylotypes. Phylotype I is reported to contain all strains belonging to biovars 3, 4 and 5, isolated from Asia. Phylotype II contains all race 3 strains pathogenic to potato, race 2 that is pathogenic to banana, biovar 1 and 2 strains and 2T (a subgroup of biovar 2 for tropical areas) isolated from America. Phylotype III comprises of strains belonging to biovars 1 and 2T from Africa and surrounding islands. Phylotype IV is more heterogeneous, with biovar 1, 2 and 2T strains from Indonesia, strains isolated in Australia and Japan (Champoiseau *et al.,* 2009). A sequence analysis of the endoglucanase *(Egl*) and MutS) has been used in classification of phylotypes (Rodrigues *et al.,*

2012). Classification based on molecular analysis of partial sequences of the spacer region 16S-23S rDNA(ITS) and the house keeping genes Endoglucanase and Muts is also described by Fegan and Prior (2005).

2.6 Effect of *Ralstonia solanacearum* in potato plants

One of the most significant diseases in potato production is bacterial wilt, caused by *R. solanacearum* Race 3 Biovar 2. It causes wilting, stunting and yellowing of the foliage, leaves bend downward showing leaf epinasty, adventitious roots grow in the stems, and narrow dark stripes corresponding to the infected vascular bundles beneath the epidermis are observed (Alvarez *et al.,* 2010). Bacteria wilt development is favoured by wet and warm soils, contaminated soil, heavy clay soils, susceptible cultivars and contaminated seed among other factors (Potatoes South Africa, 2015).

Ralstonia solanacearum primarily infects host plants through their roots, entering through wounds formed by lateral root emergence or by root damage caused by soil borne organisms such as nematodes. The bacterium can also enter plants by way of stem injuries from insects, handling or tools. Once bacteria infect the roots or stems, they colonize the plant through the xylem in the vascular bundles. The pathogen is most severe on plants at temperatures between 24°C and 35°C and decreases in severity when temperatures exceed 35°C or fall below 16°C. It is transmitted by contaminated soil, water, equipment, personnel or by transplantation of infected plants, tubers, or cuttings. The pathogen has been found to cause wilt in potato and other cops such as geranium, tomato, eggplant and woody nightshade (Ibrahim *et al.,* 2005; McKellar and Snover-Clift, 2007).

2.7 Effect of soil fertility on bacterial wilt in potato production

Poor soil fertility is among the major challenges exacerbating bacterial wilt in farmers' fields leading to a decline in potato production and yields (MoA, 2010). Continuous cultivation on agricultural land causes decline in organic matter that affect crop performance, increase nematodes infestation which facilitate infection of crops by bacterial wilt (Akanni and Ojeniyi, 2008). Potato yield and quality is strongly related to soil fertility, being a result of pre-crop investment into legumes and organic residues, intercropping and use of animal manures, green

manures or inorganic fertilizers. However, in practice humus contents of soils are low thereby affecting potato yields and quality negatively.

Improved rotations using crops with a high production of organic matter increases productivity, activates microbiological processes in the soil thereby suppressing soil borne pathogens which results to improved soil fertility and health. Potato is considered a heavy feeder and requires adequate application of essential nutrients (Powon *et al.,* 2005) especially at the tuber bulking stage and lack of the right nutrients can spoil the crop quality and reduce yield. The main macro nutrients required by the potato are N, P, K, Ca and Mg (Zaag, 1981). Micro nutrients required are Cu, Zn, B, Mn and Fe (Westermann, 2005). Nitrogen is an important factor required in obtaining high yield and it is often a limiting factor in sandy and loamy soils. Nitrogen deficiency in potatoes results in stunted growth, yellowing of the older leaves, dieback of the vine, and poor yields. Low nitrogen also accentuates diseases such as early blight and Verticillium wilt. A deficiency of N causes very small tubers, high sugar levels, low dry matter, over-mature tubers and increase in disease susceptibility (Blumenthal *et al.,* 2008). An excess of nitrogen may delay the onset of tuber set, increase knobby potatoes, and promote excess vine growth (Rosen, 1991). Low pH is one of the factors that encourage the multiplication of *R. solanacearum* Race 3 biovar 2 (R3b2) (Janse, 1996).

Most small scale farmers do not use commercial fertilizers due to the high cost and instead practice potato production without the fertilizers or rely on manure. Cow, sheep, chicken manure and pig slurry are among the most common animal manure used for potato production. A study on the effect of poultry manure, broiler litter, poultry droppings, pig slurry, goat and cow manure on potato tuber yield showed that poultry manure gave the highest yield (15 t /ha) compared to no manure (6.7 t/ha) (Wijewardena, 2000). Use of organic manures and rotation with crops such as cowpea, soybean, sorghum and pearl millet with high above ground matter also contributes to improved microbial activity and improved soil structure. The leguminous crops fix atmospheric nitrogen in the soil and are also considered important for crop rotation. A study on crop rotations for potato showed that rotation with shallow rooted crops such as carrots and onions is ideal for potato production since they do not compete for nutrients at the potato root depth (Lemaga *et al.,* 2001).

2.8 Management of bacterial wilt of potato

2.8.1 Overview of management options

Some of the major challenges facing management of bacteria wilt in small scale farming are the informal seed potato systems. Quarantine as a control measure is not feasible due to uncontrolled movement of seed and porous borders that allows for illegal importation of seed potato (Muthoni *et al.,* 2010). Chemical control has been used especially in large scale farming by the use of chemicals such as algicide (3-{-indolyl} butanoic acid, use of fumigants such as metam sodium, actigard, vapam and chloropicrin (Yuliar and Koki*,* 2015). Some of the chemicals found to have some efficacy in control of bacterial wilt are Actigard, vapam, methyl bromide and choropicrin. Chemical control is not environmentally friendly to the naturally occurring flora and fauna in the soil. It also has negative impacts to the users and the surroundings. It is unaffordable to the small scale farmers and its application is also expensive (Champoiseau *et al.,* 2010).

Some wild potato species are resistant or tolerant to the disease and have been used in development of cultivar resistant studies (Potatoes South Africa, 2015). Some of the cultivars developed with some level of resistance are always limited due to linkage with undesirable traits (Champoiseau *et al.,* 2010). High level of resistance to bacterial wilt have been identified in a *Solanum phureja* species (Sequeira and Rowe, 1969). Resistance to race 1 and tolerance to race 3 of *R. solanacearum* was also found in *Solanum stenotomum* (Fock *et al.,* 2005). Development of potato cultivars with a *Solanum phureja* and *S. demissum* showed resistance to *R. solanacearum* in seven countries. Resistance to *R. solanacearum* is generally limited by the race and strain diversity of the pathogen that makes it difficult to utilize in different agro climatic conditions (French and De Lindo, 1982; Champoiseau *et al.,* 2010). Cultivar resistance to bacterial wilt has been shown to be minimal in commercial varieties as indicated by a survey undertaken in Kenya with five common potato varieties. None of the potato cultivars used in the study was found to be resistant to bacterial wilt (Felix *et al.,* 2010).

Use of biological control agents (BCAs) is based on competition for nutrients and space, antibiosis, parasitism and induced systemic resistance. Some of the challenges facing the use of BCAs is inconsistent colonization, narrow host range, degree of suppression is too low to warrant for commercialization, instability in their effectivity in terms of time, storage and

application (Whipps and Gerhardson, 2007; Wei *et al.,* 2011; Chen *et al.,* 2014). The use of biological control agents has also been researched *in vitro* and *in vivo* and their effectiveness demonstrated with bacteria such as *Pseudomonas* spp. and *Bacillus* spp. (Ramesh and Phadke, 2012; Kurabachew and Wydra, 2013). They have been shown to suppress disease incidence and also increase the survival rates of plants (Smith, 2000).

Intercropping and crop rotation are also farming practices that have been widely researched on and practiced in suppression of bacterial wilt (Lithourgidis *et al.,* 2011; Amb and Ahluwalia, 2016). Utilization of more than one cultural practice in disease management and phytosanitation has been widely used and recommended (Gildemacher *et al.,* 2009). Recommended cultural practices include use of clean seed, quarantine measures, crop rotations, and use of cover crops, treatment of surface water, soil amendment measures and integrated application of these practices (Champoiseau *et al.,* 2010).

2.8.2 Role of crop rotation in management of bacterial wilt

Among many cropping systems that are practiced to enhance soil fertility and curb soil diseases, crop rotation is one of the most widely practiced. Crop rotation has been based on several principles such as use of non-host crops, crops with suppressive effect, N fixing plants and plants with high residue organic matter. A five year crop rotation trial of potato with wheat- maize-potato reduced bacterial wilt disease incidence to 6.3% compared with potato monoculture (80%) over the 5 years (Vema and Shekhawat, 1991). The same study showed reduction of tuber rot from 62.2% to 9.5%. Reduction of *R. solanacearum* in the soil was also demonstrated in a maize-tomato rotation which resulted in reduced bacterial wilt incidence on tomato. Despite maize being a non-host to *R. solanacearum*, it has been reported to encourage growth of antagonistic bacteria known as *Pseudomonas cepacia* (Elphinstone and Aley, 1993). The efficacy of crop rotation is also determined by the type of soil, soil pH, soil moisture, weather and other abiotic factors and therefore crop yield and disease incidence may vary from one location to another and from one season to another as indicated by Adebayo and Ekpo (2006)**.**

Utilization of different crops in rotation regimes is also used with the objective of incorporating the plant biomass into the soil as green manure. Green manure legumes when used as relay intercrop into a maize bean rotation system increased the yield of the subsequent maize crop

(Nyambati *et al.,* 2009). This indicates the significance of residual effect of the green manures to the subsequent crops grown. The presence of bacteria that aid in transforming atmospheric N to ammonia available to plants is another concept that is utilized in crop rotation patterns. These bacteria include *Rhizobium* and *Bradyrhizobium* species that establish symbiosis with leguminous plants. Other non-symbiotic free living N fixing bacteria that enhance the growth of other plants such as wheat, sorghum, maize, rice include *Azospirillium, Azoarcus, Azotobacter, Burkholderia* species*,* (Perez-Montano *et al.,* 2014).

Allelopathy due to crop exudates is also another factor that is exploited in crop rotation systems. Phytotoxins produced by plants are reported to reduce the establishment, growth and survival of susceptible plants. This suppresses growth of targeted weeds, reduced competition of resources and better growth of target crops (Shukla *et al.,* 2011). The use of cover crops and retaining of crop resides on the soil surface after harvesting influences the micro climate and this can have a positive or negative effect on the pathogen population depending on the optimum environment of the pathogens. It improves the soil environment by reducing evaporation and increasing reflectivity which facilitates germination and growth of subsequent crops. Retaining crop residues may reduce the crop stand depending on the quantity and quality of crop residues. It is recommended to leave crop residues undisturbed and delay incorporation when working with vegetables to slow down mineralization since some vegetable crop residues are reported to mineralize rapidly especially during warm seasons (Agneessens *et al.,* 2014).

Utilization of more crop rotation seasons and the use of different crops in the rotations have proved useful in reducing the effects of *R. solanacearum* due to withdrawal of the host. Lemaga *et al.* (2001) concluded that a crop rotation of more than two different crops grown in succession is a more effective practice in control of the pathogen. In this study, potato-sweet potato-potato had the least bacterial wilt incidence in the one crop rotation while the potato-beans- maize-potato rotation showed the lowest bacterial incidence in the two crops rotation. In the same study, rotation of potato with crops such as carrots, onions and peas produced a high crop yield (t/ha) compared to rotation with beans and finger millet. A one season rotation of potato with cabbage, beans and maize showed that cabbage had significantly higher marketable yields to the subsequent potato crop compared to the other crops (Nyangeri *et al.,* 1984). Rotation of maize with potato in this study also reduced bacterial wilt in the one season crop rotation. Other non-

host crops to *R. solanacearum* that have been recommended include rice, yam, chive, garlic, bulb onion, spring onion, asparagus, cabbage, Ethiopian mustard, canola, lettuce, pea, yard long bean among others (Wang and Lin, 2005).

Among crops used for rotation, some have been known to produce exudates that are suppressive to *R. solanacearum*. These plant exudates have secondary metabolites that include; Nitrogen and sulphur-containing secondary compounds such as alkaloids, terpenes, glucosides, phenolic compounds such as flavonoids, anthocyanins, tannins and others. Terpenes include limnoids and saponins. Among crops known to have nitrogen containing secondary metabolites that are useful in defense mechanism include the cabbage and radish. Legumes are also reported to contain isoflavonoids and saponins (Bais *et al.,* 2006). Chinese chive, garlic and fodder radish (*Raphanus sativus*) have been reported to have suppressive effects and to produce antibacterial effects to *R. solanacearum* in tomato (Jing, 1999). However, despite the significant role played by the above crops in suppressing bacterial wilt, research has also shown that some of these crops allow high incidence of bacterial wilt in crop rotation (Lemaga *et al.,* 2001). Among other agronomic reasons, the possibility of latent infection within these crops could lead to an increase in the bacterial population with time.

2.8.3 Effect of soil amendments on bacterial wilt and potato yield

Organic manures have been used as soil amendments to suppress *R. solanacearum*. Soils amended with 5 and 10% farm yard manure (FYM), 10% cocopeat and 1% green compost were found to completely inhibit infection of tomato by *R. solanacearum* in comparison to the control where disease severity was high (Yadessa *et al.,* 2010). This was attributed to a high microbial activity in the soil and improved physical and biochemical characteristics of the soil. The study also showed that FYM did not only suppress the pathogen but also caused an increase in tomato yield (Yadessa *et al.,* 2010). Sun hemp and *Tithonia diversifolia* as green manures were also reported to suppress *R. solanacearum*. *Tithonia diversifolia* (10t/ha) applied as green manure used in combination with other organic manures were found to reduce bacterial wilt incidence in potato cultivation in the Sri-lanka soils (Kelaniyangoda *et al.,* 1995). NPK-amendment to Egyptian sandy soil was found to reduce potato wilt severity by almost 100%. In this study the researchers also observed that bacterial suppression by the organic and inorganic manures varied with the soil types. The type of soil has also been found to be an important factor in affecting the

populations of the pathogen and disease severity. A study by Messiha *et al.* (2007) showed that populations of *R. solanacearum* were higher in bulk soil and rhizosphere of potato plants grown in organic sandy soils from the Netherlands, disease severity was highest in Dutch sandy soils compared to the other soils in the study and disease suppression was more in Dutch clay soils compared to Dutch sandy soils. Not all organic amendments have been found to suppress bacterial wilt. Islam and Koki (2004) reported that bark compost, coffee manure and pig manure showed higher bacterial wilt incidence in tomato compared to poultry and FYM application which resulted to a lower wilt incidence.

Use of organic materials in management of soils is also widely practiced in crop production. Several innovative ways of utilizing organic materials have been developed such as TakaTaka compost. An award winning company "Takataka Solutions" is utilizing organic wastes from the fruit/vegetable markets in Nairobi, Kenya and composting them to products that farmers can use. The company produces compost resulting from controlled biological decomposition of organic materials (www.takatakasolutions.com). Other organic fertilizers available in the market include Neem gold; which contains Azadirachtin, Nimbin and Nimbidin that imparts incredible pesticidal properties such as; control of nematodes, optimization of soil micro flora, etc. Application of neem residues also improves the water-holding capacity of soil, reduces leaching and regulates nitrogen when applied with NPK fertilizers. When neem was used with vermicompost and FYM, it increased plant height and tillers per sorghum plant (DARE/ICAR, 2009).

Inorganic fertilizers also play a vital role in crop yield and disease suppression. Potassium for example is important in cellular division and formation of energetic structures and reduces susceptibility to plant diseases whereas phosphorus facilitates transport of sugars, stomata control and is a co factor of many enzymes. Twenty tons Farm yard manure + 100 kg P_2O_5/ha^{-1} was shown to give high tuber yields in comparison to control treatment and treatment with 50 kg P ha^{-1}, which gave the lowest total tuber yield according to Powon *et al.,* (2005). Further studies confirm that a combination of organic and inorganic manures is important in achieving high potato yields. Treatments of Sesbania with NPK resulted in significantly higher marketable yields of ware size potatoes compared to other treatments where organic or inorganic manures were used separately (Lemaga *et al.,* 2001). Reinforcement of inorganic fertilizers with organic

or green manures according to research is a promising strategy in control of *R. solanacearum* (Kelaniyangoda *et al.,* 1995; Lemaga *et al.,* 2001).

Many control strategies have been evaluated to control bacterial wilt in most of the crops it affects. However, an integrated approach is recommended; one that would involve biological control agents, organic matter such as organic compounds, compost or plant residues (Yuliar and Koki*,* 2015).

CHAPTER THREE

STATUS OF POTATO BACTERIAL WILT IN POTATO PRODUCING WARDS OF NAKURU COUNTY, KENYA

3.1 Abstract

A survey was carried out in nine Wards of Nakuru County, Kenya between October and December 2012. The purpose of the survey was to provide information on the occurrence of bacterial wilt and contributing factors to the disease development in the County. These factors were determined through observation and farmers' interview. Descriptive statistics were used to determine percentages and frequencies of data collected. Chi-square tests and spearman's correlation coefficient analysis were used to determine significance of the data and examine relations between different variables respectively. GPS coordinates of the fields were recorded using Geographical Positioning System (GPS). Biochemical determination of biovars of 20 *R. solanacearum* isolates collected during the survey was done on the basis of carbon utilization in disaccharides and oxidation of hexose alcohols. Bacteria wilt prevalence in the County varied from 100 to 35.7 percent and was found to be spread across all the Wards surveyed. The incidence varied from 0 - 41% in the farms surveyed. There was a negative relationship between altitude and bacterial incidence [x^2 (36, N=111) =78.6, $p<0.01$]. Bacterial wilt incidence reduced as altitude increased [r_s= -0.30, (n=111, $p<0.01$)]. Bacterial wilt symptoms on plants were more prevalent from the principal growth stage 6; first open flowers to end of flowering. Major contributing factors to bacterial wilt in the region included; seed source, potato variety, and lack of seed renewal. Two biovars were identified, biovar 2 and 3; the presence of biovar 2 and 3 indicate the need for farmers to weed out purslane (*Portulaca oleraceae*) and nightshades (*Solanum dulcamara*) weeds from their farms since they may serve as alternative hosts to the strains. More capacity building of farmers should be done to encourage sourcing of seed from recognised institutions or out growers. Promotion of positive seed selection after principal growth stage 6 should be also encouraged.

3.2 Introduction

Potato (*Solanum tuberosum* L.) is an important food crop in Kenya's food security and ranks second to maize. In Kenya, most potato production is done on the slopes of Mount Kenya, along the Aberdare ranges, Mau ranges and some highland regions in Nyanza, Western and Trans – Nzoia Counties (Kaguongo *et al*., 2013). Several surveys have been undertaken in Kenya on the prevalence and incidence of bacterial wilt in several potato producing zones in Central and North Rift Valley and have shown bacterial wilt to be an important potato disease (Ateka *et al.,* 2001; Nyangeri, 2011; Kwambai *et al.*, 2011). In the North Rift region of Kenya, bacterial wilt is highly prevalent in Keiyo and Uasin Gishu as observed by Kwambai *et al.* (2011) and Kwambai *et al.* (2011). A study on the occurrence of *R. solanacearum* in some of the major potato production zones of Kenya showed that 58.7 % of the farms assessed were infested with *R. solanacearum* (Nyangeri, 2011). Conducting baseline, risk and impacts assessments to quantify problems on the ground provides strong foundation for improved decision making for stakeholders and demonstrates outcomes and impacts. Integrating state of the art scientific knowledge with farmers' knowledge also improves on disease management. Surveys are therefore crucial to provide such knowledge which is lacking or out-dated (Schulte-Geldermann, 2013). Bacterial wilt status varies from one region to another and therefore site specific assessment of a region is crucial to understand the significance of the diseases and the contributing factors.

Geographic distributions of pathogens are highly influenced by factors such as availability, susceptibility and abundance of the host and suitability of the climatic conditions (Shaw and Osborne, 2011). Availability of the pathogen, host and a favourable environment in potato production areas encourage bacterial wilt development. *Ralstonia solanacearum* race 3 biovar 2 causing bacteria wilt in potatoes is found commonly in areas of higher elevations (Priou *et al*., 1998). Five races and biovars have been described so far, and Africa is the continent where the highest diversity is found (Álvarez *et al.,* 2010). A study in Kenya showed Biovar 2 was more common in the potato production ecosystems compared to biovar 3 which was found in the UM3 zone of Central province and specifically in Muranga (Nyangeri, 2011); biovar 2 was found to infect potatoes while biovar 3 was isolated from eggplants (Nyangeri *et al.*, 1984).

Information on the geographic distribution of *R. solanacearum*, host availability, suitability of climatic conditions and farming practices is vital in disease spread and for national risk preparedness in avoiding losses in produce. Such information gives the relevant stakeholders a better focus in improving the farmers' practices to increase potato yield. Despite Nakuru County being a major potato production zone (Kaguongo *et al.,* 2009), little information is available on the geographic distribution of potato bacterial wilt in the region. The purpose of this survey was to provide comprehensive information of the potato bacterial wilt status of Nakuru County. The study describes the spatial variation of bacterial wilt incidence and prevalence in the County. It points out areas of high risk in order to allow for appropriate management strategies to be employed. It also identifies key farming practices that could be contributing to the bacterial wilt status in the area. It provides information on biovars identified in the region and the hosts from which they were isolated from. This knowledge is useful for site-specific management of bacterial wilt in the County.

3.3 Materials and Methods

3.3.1 Determination of prevalence and incidence of bacterial wilt

A survey was carried out in five of eleven Sub-Counties in Nakuru County, Kenya namely; Mau Narok and Mauche Wards in Njoro Sub-County, Molo and Elburgon Wards in Molo Sub-County, Kamara in Kuresoi North Sub-County, Keringet, Kuresoi and Olenguruone Wards in Kuresoi South Sub-County and Bahati in Bahati Sub-County which had potato production during the short rains of October to December 2012. It was carried out in 145 potato farms. The sample size was estimated according to the formula below by Cochran (1963). A confidence level of 92% and a precision level of ±7% were applied; the estimated proportion of potato farmers in the short rains in the surveyed wards was 60%.

$$n_0 = \frac{z^2 pq}{e^2}$$

Systematic random sampling was done where at least fifteen potato fields were selected at a distance of not less than 7 kilometres in each potato-producing Ward to determine the disease prevalence and incidence. The sampling interval was calculated based on the systematic random sampling formula below according to Ahmed (2009), however, in this case, the approximate total

area of the wards surveyed (989 km^2) was calculated against the total number of sampling units that resulted to the 7 kilometres sampling interval.

$$Sampling\ interval(K) = \frac{Population\ size\ (N)}{Number\ of\ sampling\ units\ (n)}$$

Disease prevalence was determined by counting the number of potato fields with bacterial wilt expressed as percentage of the total number of fields assessed in each Ward according to the formula below (Mehotra and Aggarwal, 2003).

$$Disease\ Prevalence = \frac{Number\ of\ fields\ with\ bacterial\ wilt}{Total\ number\ of\ fields\ assessed}\ x100$$

Disease incidence was determined through observation of symptomatic plants in the field. Four plots of 6 rows by 10 plants per row were demarcated in each field and bacterial wilt symptomatic plants counted against the total number of plants in each plot. Bacterial wilt incidence was calculated using the formula by Mehotra and Aggarwal, (2003).

$$Bacterial\ wilt\ incidence = \frac{Number\ of\ plants\ with\ symptoms}{Total\ number\ of\ plants\ assessed}\ x100$$

3..3.2 Survey protocols

A semi-structured questionnaire and personal observation were used to collect data on factors influencing bacterial wilt status in the area (Appendix 1). The questionnaire was pretested before data collection for clarity of questions and administration time before being administered to 66 households in the surveyed wards. GPS coordinates of each field sampled were recorded using Geographical Positioning System (GPS) (Garmin eTrex 30). The GPS coordinates were used to develop elevation maps showing the bacterial wilt occurrence and potato production zones using ArcGIS software, version 9.0 (Esri Company) and ERDAS IMAGINE 9.1. The elevation maps were overlaid with the County boundaries, potato production zones and sampling sites showing fields with and without bacterial wilt.

3.3.3 Biochemical characterization of *Ralstonia solanacearum* isolates collected

A total of 32 isolates were obtained from tubers of infected potato plants and roots of common weeds within the farms. The diseased tubers and roots were collected from farmers' fields in five

sub Counties. Two tubers from each symptomatic plant were collected and put in a paper bag and placed in portable coolers. Roots of Solanaceous plants at the borders of the farms and along the fences were also collected. They were also placed in paper bags and coolers before being transferred to the laboratory for isolation. Isolation and culturing was done according to the method described by Kinyua *et al*. (2014) with modifications. The tubers and roots were washed under running tap water. The stolon ends of the infected tubers were cut and roots were immersed in 70% ethanol for 3 minutes, and then rinsed in sterile water. They were soaked in 10 millilitres sterile distilled water for 60 seconds with constant stirring with a sterile glass rod to get the bacterial suspension. The bacterial suspensions from individual isolates were cultured on Casamino Acids-Peptone-Glucose (CPG) media for 48 hours (Appendix 6). Bacterial colonies which were cream-colored, irregularly shaped and highly fluidal were harvested and suspended on sterile distilled water to make a bacterial suspension.

Determination of biovars of *R. solanacearum* from the diseased samples collected during the survey was done on the basis of carbon utilization in disaccharides (cellobiose, lactose and maltose) and hexose alcohols (dulcitol, mannitol and sorbitol) according to the method described by Denny and Hayward (2001). The hexose alcohols were autoclaved while the disaccharides which are heat labile were filter sterilized. Ten millilitres of each given carbon source solution was added to 90ml molten basal medium (Appendix 4) and cooled to about 50^{o}C. After mixing, the medium was dispensed in 4 ml aliquots into previously sterilized test tubes (150mm x 10mm size). The media was stab-inoculated in duplicates with bacterial cultures of pure isolates and plugged with non-absorbent cotton. Two controls were set-up; one without any carbohydrate (salicin) and another control with dextrose. The cultures were incubated at 30^{o}C and examined after 2, 7 14 and 21 days for the utilization of the disaccharide and oxidation of the alcohol expressed by the change in the medium colour from green (neutral pH) to yellow (acidic pH) (Figure 3.1).

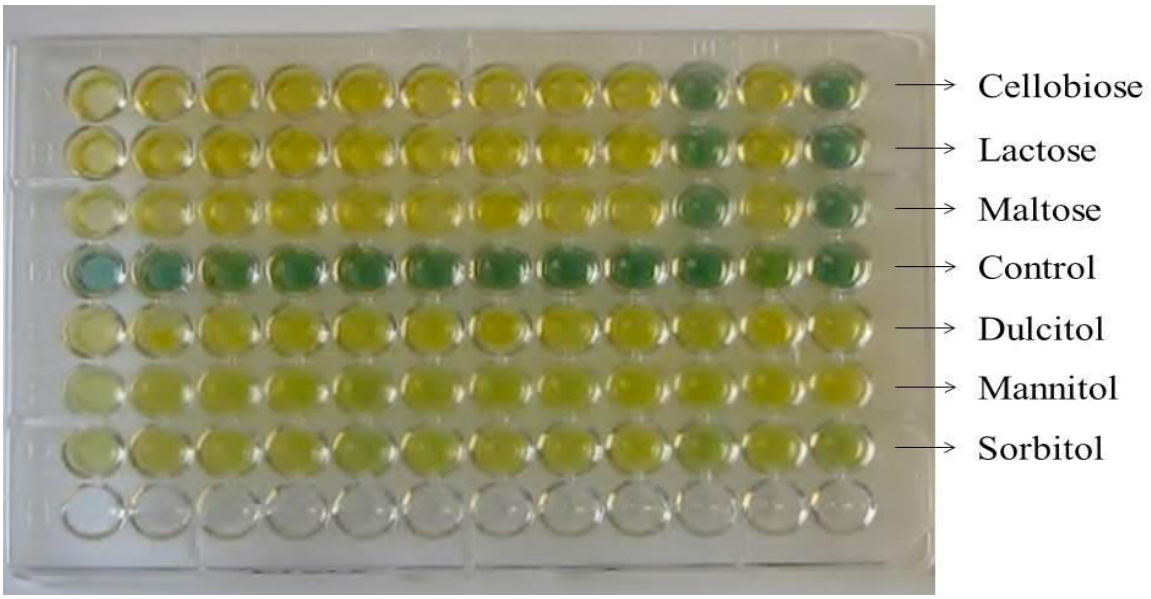

Figure 3. 1: Carbon utilization test showing a positive result (yellow colour) and a negative result (green colour). Source: Kinyua *et al.* (2014)

3.3.4 Data analysis

Descriptive analysis was used to determine percentages and frequencies of data collected on economic and social aspects influencing bacterial wilt status in the area. Chi-square test was used to determine significance of the data and spearman's correlation coefficient analysis was performed to examine relations between the different variables. This was done using IBM SPSS software statistics version 20. Data analysis for GPS data was done using Stata Statistical Software Release 11 (StataCorp LP).

3.4 Results and Discussion

3.4.1 Distribution of potato bacterial wilt in Nakuru County

The survey was carried out in Wards where potato production was the main crop in the short rains season. Potato production was concentrated along the Eastern and Western Mau Forest and along Bahati Forest (Figure 3.2*).* Production of potato was found to occur in nine Wards namely; Mau Narok, Mauche, Kuresoi, Keringet, Kamara, Olenguruone, Molo, Bahati and Elburgon. Most of these Wards are found in the high altitudes (2353-2942m asl) with an exception of Bahati Wards where potato production was in the lower highlands (2032-2344m asl).

3.4.1.1 Distribution of bacterial wilt in Wards

Bacterial wilt was found to be spread within all the sub Counties surveyed (Figure 3.2) at varying disease incidences. The wilt prevalence varied from 100% in Bahati Ward to 35.7% in Mauche (Figure 3.3). All the farms assessed lying at 2300 m asl and below had 100% bacterial wilt prevalence (Figure 3.4). Bacteria wilt mean prevalence in the Wards surveyed was 69% while the mean incidence was 5%. Therefore the null hypothesis indicating that bacterial wilt is not prevalent in Nakuru County was rejected. Bacterial incidence was highest in Olenguruone and Mau Narok Wards with 9.9% and 9.35, respectively. The lowest incidence was in Keringet and Molo Wards with 2.8% and 2.9%, respectively. The high disease prevalence in Bahati, Kuresoi and Olenguruone Wards may be exacerbated by spread of the disease due to seed movement within the Wards and from across the neighboring Counties. A previous study showed that Bomet County which borders Kuresoi and Olenguruone Wards had a bacterial wilt prevalence of 91%, while Nyandarua County which borders Bahati Ward was reported to have a bacterial wilt prevalence of over 50 % (Ateka *et al.,* 2001; Gildemacher *et al.,* 2009). Bacterial wilt is considered a threat to potato production in Kenya and neighboring countries such as Uganda as observed in a survey carried out in the year 2004-2005. This survey reported that 71% and 85% of the farmers in Kenya and Uganda respectively considered bacteria wilt to be the most important disease in potato production (Gildemacher, *et al.,* 2009). Another survey by Kaguongo *et al.* (2013) undertaken in ten major potato growing Counties of Kenya showed that bacterial wilt was also the most prevalent potato disease affecting 77% of potato farmers.

The observed high disease prevalence was due to the informal seed systems especially the seed sources in areas such as Olenguruone, Mau Narok, Bahati and Kuresoi (Figure 3.3 and 3.4). It was evident that most farmers in the County used their own seed or sourced from their neighbor (Figure 3.6) which was found to be a major contributing factor in the spread of bacterial wilt as also observed by Muthoni *et al.* (2010); Kaguongo *et al.* (2013); Wang'ombe and Van Djik (2013).

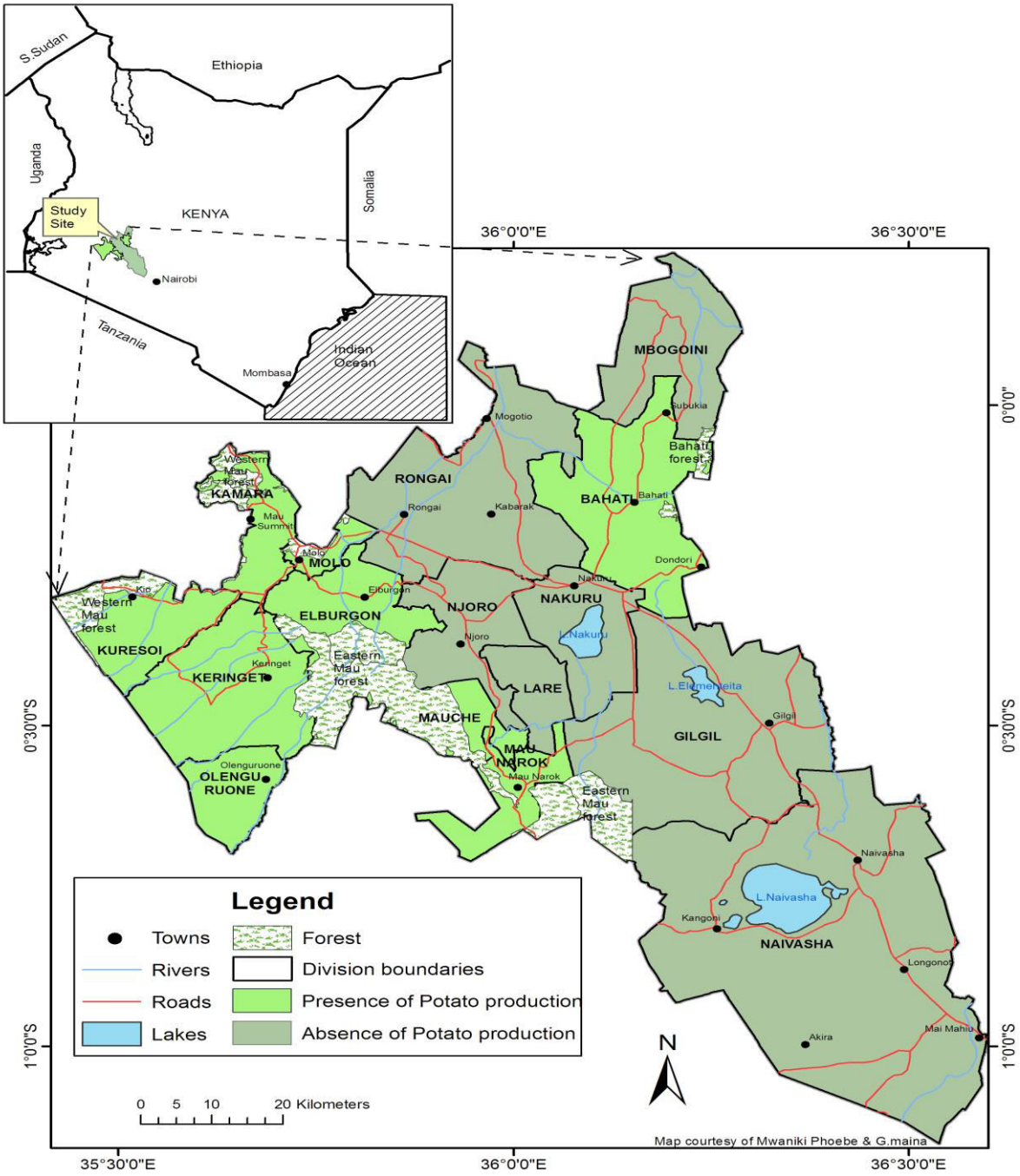

Figure 3.2 Surveyed Wards with potato production during the short rains of October-December 2012 in Nakuru County

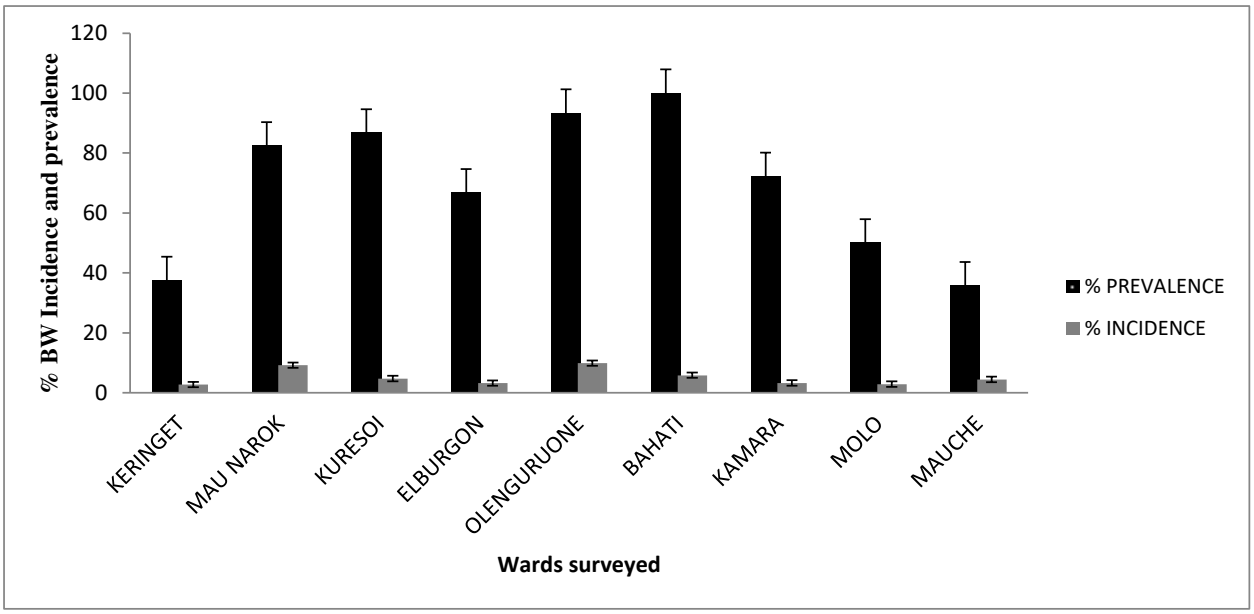

Figure 3.3: Bacterial wilt prevalence and mean disease incidence across nine Wards in Nakuru County, Kenya in the short rains season of year 2012

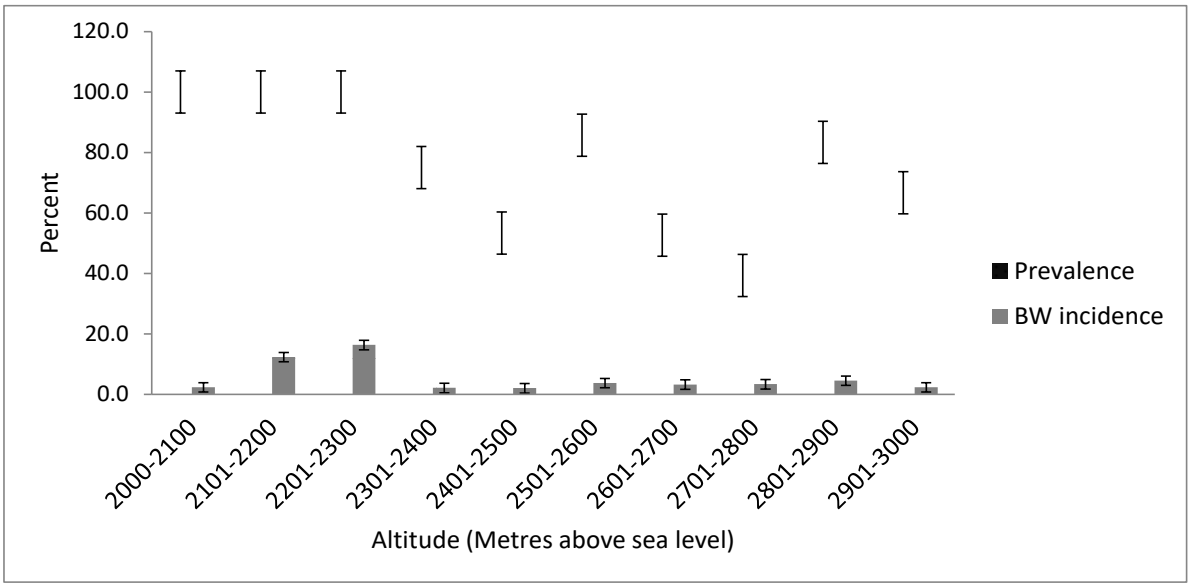

Figure 3. 4: Percent bacterial wilt prevalence (%) and disease incidence across altitude in nine Wards of Nakuru County, Kenya in the short rains season of year 2012

3.4.1.2 Bacterial wilt status across elevations

A chi square test showed that there was a significant relationship between altitude and bacterial incidence at [x2 (36, N=111) =78.6, p<0.01], (rs= - 0.30, n=111, p<0.01)]. This indicated a reduction in bacterial wilt with an increase in altitude (Table 3.1). The disease incidence in farms surveyed decreased beyond the altitude of 2300m asl, however the farms still had bacterial wilt distributed across the farms up to the higher altitudes up to 2942 m asl (the highest altitude sampled point in the study) (Figure 3. 5). Bacterial wilt has been reported to also occur in high altitudes as reported by Ateka et al. (2001). The prevalence and incidence observed in the high altitude may be due to infiltration of infected seed from the medium altitude areas where potato production is more common. Ralstonia solanacearum is reported to survive in soil temperatures as low as 4^{o}C as indicated by Milling *et al.* (2009) and Sullivan *et al.* (2013) and therefore the ability of this pathogen to survive in high altitude areas. However its virulence is reported to be low in temperatures below 16^{o}C (Ibrahim *et al.,* 2005; McKellar and Snover-Clift 2007). This may explain the low disease incidence observed in higher altitudes compared to altitudes below 2300metres above sea level (Figure 3.5).

3.4.2: Expression of bacterial wilt symptoms

Bacteria wilt symptoms were apparent in potato plants at the principal growth stage of 6; first open flowers to end of flowering (BBCH-Scale 60) onwards (Appendix 9, Figure 3.6). The BBCH identification keys of potato applied in this study are described by Hack *et al.* (1993). The symptoms of bacterial wilt were evident at an early principal growth stage 2 (formation of basal side shoots below and above the soil surface) and this could be attributed to high pathogen population or heavily infected seeds. However symptoms were not frequently observed at 3, 4 and 5 i.e leaf development, main stem elongation, tuber formation and inflorescence emergence, growth stages respectively. This gap in symptom expression may be attributed to the early death of the infected young plants. Symptomatic plants were frequently observed from the inflorescence emergence onwards. This is an important lead to the stage at which positive or negative selection of seed should be done in the farmer's fields

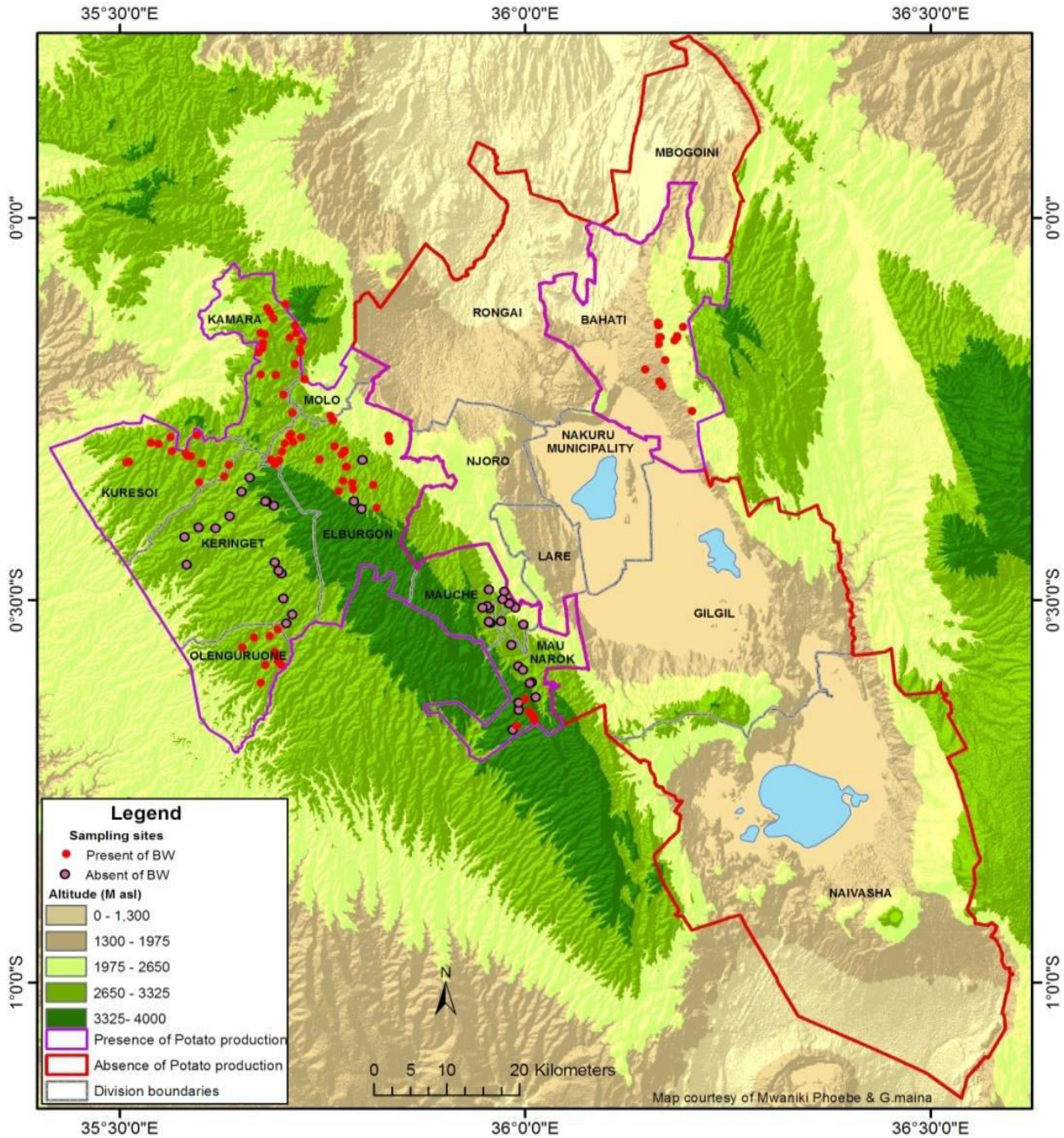

Figure 3. 5: Distribution of bacterial wilt in potato producing Wards in Nakuru County during the short rains of October – December 2012

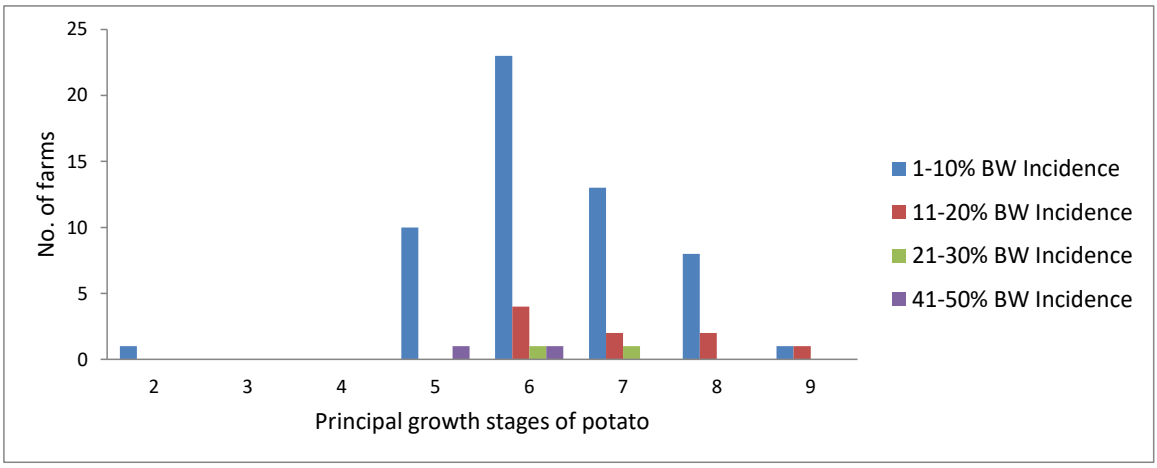

Figure 3. 6: Expression of bacterial wilt symptoms at different growth stages of potato
BW: Bacterial Wilt

3.4.3: Factors contributing to bacterial wilt status in Nakuru County

3.4.3.1 The potato biological characteristics: The most common potato varieties that were grown in the area included Shangi, Tigoni and Kenya Karibu. Other varieties that were grown in the area were Asante, Kenya mpya, Nyayo and Desiree (Figure 3.7) The findings indicated that 70.6 % of the farmers produce Shangi potato variety and most of the farmers grew other different varieties in their farms. Variety "Shangi" was very common with farmers across the sub Counties. Despite the farmers reporting it to have a short growing season and high yields, they also reported it to be susceptible to bacterial wilt. The susceptibility of Shangi has also been reported in the policymakers guide to crop diversification (FAO, 2013). The availability of a susceptible cultivar or a variety is very significant in the proliferation of any disease. The potato variety may be a contributing factor to bacterial wilt spread in the area since the availability of the host; its susceptibility and abundance contribute significantly to geographic distribution of pathogens (Shaw and Osborne, 2011). Availability of quality seed is reported to be limited in East Africa as reported by Schulte-Geldermann (2013). This is aggravated by long conservative cycles of producing certified seed by national programmes. Some of the seed potato varieties used by farmers have degenerated or lost resistance to diseases (Schulte-Geldermann, 2013). This was confirmed by continuous recycling of farmers own seed as observed in this study. Cultivar resistance to bacterial wilt has also remained a challenge to date as indicated in several studies (Felix *et al.,* 2010; Gastelo *et al.,* 2014). A survey undertaken in Kenya with five common potato varieties grown by farmers found that none of the potato cultivars used in the study was resistant to bacterial wilt (Felix *et al.,* 2010). Some cultivars resistant to bacterial wilt

have been developed in studies by French (1985); however this resistance has been limited by the races and different agro-climatic conditions (French and De Lindo, 1982).

Figure 3. 7: Common potato varieties grown in Nakuru County

3.4.3.2 Lack of efficient seed systems: The study indicated that most of the farmers sourced their potato seeds from their neighbors or used their own seed (Figure 3.8). Replanting tubers collected from the same farms (self-sourcing) infested with bacterial wilt increases the disease incidence in these farms. Sourcing seeds from the neighbors was also an important factor considered to be contributing to the high disease prevalence in the region because it facilitates spread of the pathogen from one field to another. This is a concern that has also been highlighted in a report by the National Potato Council of Kenya, (2013). Use of seed that is latently infected with *R. solanacearum* has been reported to contribute significantly to bacterial wilt spread (Champoiseau *et al.,* 2010). This study agrees with the findings of Schulte-Geldermann (2013) that most farmers in East Africa lack reliable seed sources and heavily rely on farm saved seed potatoes. A study carried out in Njoro and Kuresoi showed that the price of certified seed potato was a limiting factor in the use of clean seed by most farmers since it was reported to be double the price of the locally available seed. The available certified seed potato accounts for only 2% of the country's seed potato demand (MoA, 2008; Wang'ombe and van Djik, 2013) and this encourages the use of farm saved seed.

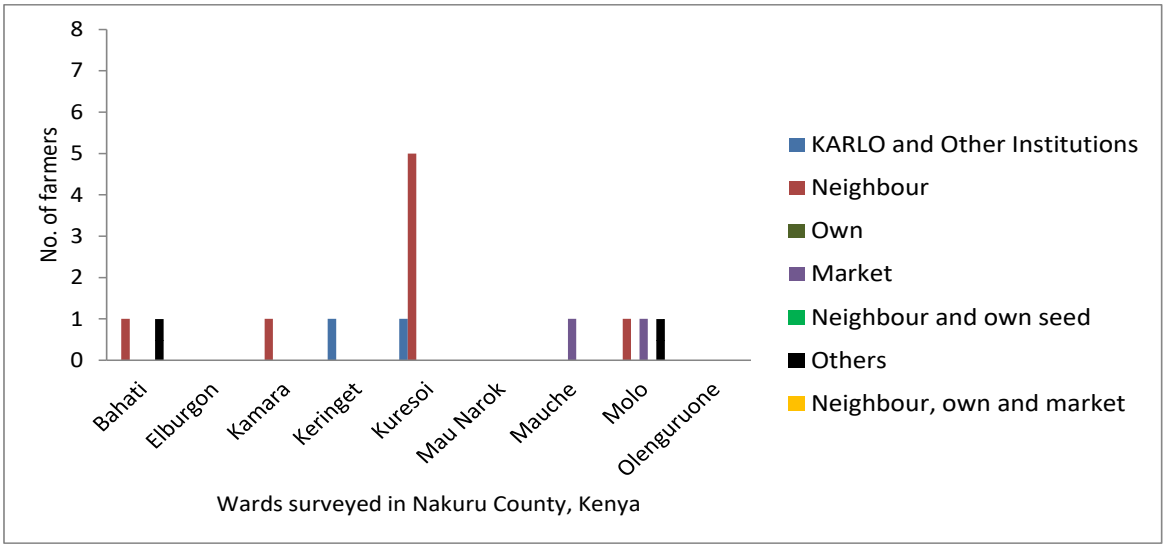

Figure 3. 8: Farmers' potato seed sources by Wards

3.4.3.3. Farmers' practices: Thirty six percent of the farmers reported that they do not change their seed and usually just sort the seed for the next planting from their previous harvest, whereas 44% reported that they source for new seed after three generations of potato production (Figure 3.9). This also contributes to the increase of bacterial wilt in these farms. Use of the same seed over several generations as observed in this study contributes to low quality and degenerated seed potato. These informal seed systems have been observed in several studies and are considered a major contributing factor to the spread of the disease (Muthoni *et al.,* 2010; Wang'ombe and Van Djik 2013; Kaguongo *et al.,* 2013).

Crop rotation was practiced by some farmers with pre-crop seasons to potato varying from one season to five seasons. There was a significant effect on the number of seasons of other crops in rotation with potato crop on bacterial wilt incidence according to the farmers information. Spearman's correlation coefficient of r_s= -0.092, n=46 indicated a reduction of bacterial wilt with an increase in the number of seasons under other crops (Table 3.1). This is a positive indicator of the contribution of crop rotation in farmers' fields. The significance of ample crop rotation is also highlightened by Lemaga *et al.* (2001) and Gildemacher *et al*. (2009). Crop rotation, being a common cultural practice with farmers in potato production, differs with the number of seasons and sequence of crops in different regions. Research carried out in Peru indicated that farmers practice crop rotation of potatoes using crops such as Mashua, Oca,

Olluco, wheat and barley and the rotation extends to 7-8 years (Raul *and* Petrescu, 2009). In the Andes, potato is usually the first crop in the rotation cycles and usually planted after cereals and before legumes (FAO, 2009).

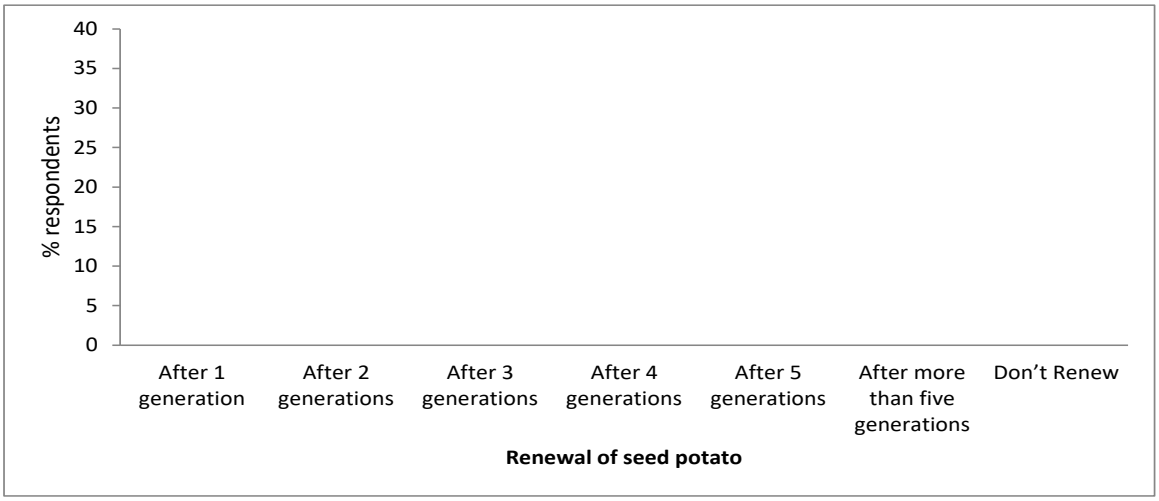

Figure 3. 9: Seed potato renewal by farmers

3.4.3.4. Level of awareness*:* Knowledge among several farmers on the significance of bacterial wilt to potato production was also found to be a contributing factor in its proliferation. Seventy eight percent of the farmers were aware that diseases can be spread through seed potato tubers while 21.3 % did not know that tubers could be a source of disease spread (Figure 3.10). Ninety three percent of the farmers indicated that they incurred losses due to bacterial wilt and 6.3 % were not aware of the significance of the disease to their produce. The farmers reported experiencing yield losses ranging from 5% to 80% due to bacterial wilt. Lack of awareness on the significance of bacterial wilt leads to farmers using latently infected seeds which introduces inoculum to new fields.

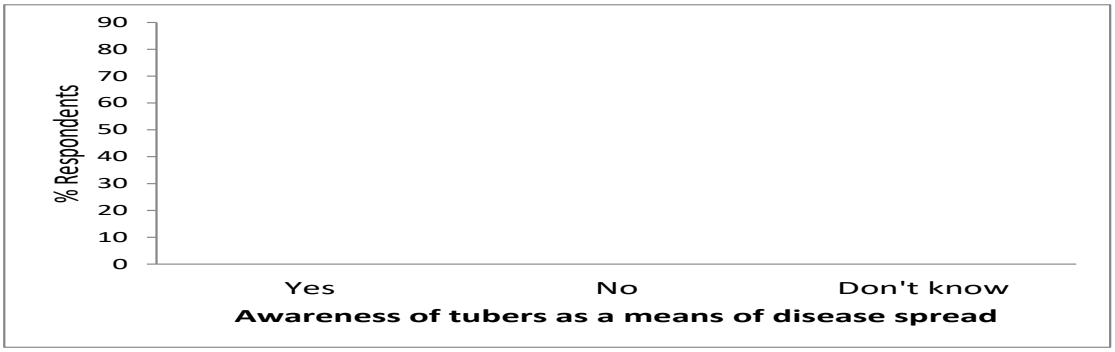

Figure 3. 10: Level of awareness of farmers that tubers can spread diseases

Table 3.1: Spearman's rho correlation coefficients for the relationships of altitude, Bacterial wilt incidence, crop growth stage, yield and acreage under potato production.

Variable	Altitude	B W I (%)	BBCH	Yield
Altitude	-	-0.289**	-0.025 ns	-0.110 ns
BW Incidence (%)		-	0.236*	0.150 ns
BBCH				-0.006 ns
Acreage under potato production	0.186*	0.072 ns	-0.026 ns	0.78**
No. of seasons before potato crop	-0.002 ns	-.092 ns	-0.255 ns	0.019 ns

**Significant at p<0.01, *Significant at p<0.05, ns-not significant

BWI: Bacterial Wilt Incidence, BBCH: Phenological growth stages of potato

3.4.4 Identified *Ralstonia solanacearum* biovars in selected Wards of Nakuru County

Twelve of the isolates collected did not indicate characteristic oxidation of the dissacharides and hexose alcohols in the biochemical characterization and therefore only those that had clear reaction are listed (Table 3.3). Sixteen (80 percent) of the bacterial wilt isolates isolated from potato and *Portulaca oleraceae* oxidized the disaccharides used; cellobiose, lactose and maltose, by changing colour of the medium from green to yellow and failed to oxidize hexose sugar alcohols; mannitol, sorbitol and dulcitol therefore identified as biovar 2. Four (20 percent) of the isolates collected from bitter sweet nightshade oxidized all the disaccharides and the hexose alcohols therefore were identified as biovar 3 (Table 3.3). Biovar 2 was isolated from potato and the bitter sweet nightshade while biovar 3 was isolated from purslane weed (Table 3.2). Biovar 2 and 3 are reported to infect a wide range of cultivated plants and weeds such as geraniums, tomatoes, sesame, banana eggplant (Priou *et al.,* 1998; Jeong *et al.,* 2007; Perez *et al.,* 2008). A study carried out in Korea, Nepal and Thai land showed that both biovar 2 and 3 infect potatoes (Dhital *et al.,* 2001; Jeong *et al.,* 2007). Biovar 3 in potato has also been associated with Race 1 (Priou *et al.,* 1998; Jeong *et al.,* 2007). Race 3 biovar 2 is reported to survive in higher elevations by Perez *et al.* (2008) as also observed in this study. The presence of biovar 3 in Kenya is also confirmed by studies done in central Kenya where it was isolated from eggplants (Nyangeri, 2011). According to the study, purslane weed was also more susceptible to biovar 3 than to biovar 2 when artificial inoculation was done. Purslane weed, also known as duck weed, is a common weed found in moist highlands and in this study it was found in elevations above 2700

m asl. It was found to be associated with biovar 3. Previous research has shown that purslane weed is also a host to *R. solanacearum* biovar 2 (Pradhanang *et al.,* 2000).

Table 3.2: Host, collection sites and altitude of isolates used in biochemical characterization

Isolate Number	Host source Scientific name	Common name	Collection site	Altitude
NCIS1	*S. tuberosum*	Potato	Kuresoi	2731
NCIS 2	*Portulaca oleraceae*	Purslane weed	Kuresoi	2752
NCIS 3	*Portulaca oleraceae*	Purslane weed	Kuresoi	2726
NCIS 4	*S. tuberosum*	Potato	Kuresoi	2749
NCIS 5	*S. tuberosum*	Potato	Kuresoi	2662
NCIS 6	*S. tuberosum*	Potato	Molo	2771
NCIS 7	*Portulaca oleraceae*	Purslane weed	Molo	2707
NCIS 8	*S. tuberosum*	Potato	Molo	2708
NCIS 9	*Portulaca oleraceae*	Purslane weed	Molo	2728
NCIS 10	*S. tuberosum*	Potato	Molo	2563
NCIS 11	*S. dulcamara*	Bitter sweet nightshade	Keringet	2331
NCIS 12	*S. tuberosum*	Potato	Keringet	2669
NCIS 13	*S. tuberosum*	Potato	Keringet	2606
NCIS 14	*S. tuberosum*	Potato	Keringet	2591
NCIS 15	*S. tuberosum*	Potato	Keringet	2714
NCIS 16	*S. tuberosum*	Potato	Njoro	2419
NCIS 17	*S. dulcamara*	Bitter sweet nightshade	Njoro	2510
NCIS 18	*S. dulcamara*	Bitter sweet nightshade	Njoro	2465
NCIS 19	*S. tuberosum*	Potato	Njoro	2339
NCIS 20	*S. tuberosum*	Potato	Njoro	2428

NCIS: Nakuru County Isolate

Table 3. 3 Classification of isolated *R. solanacearum* into biovars

ISOLATE	Maltose	Lactose	Cellobiose	Dulcitol	Sorbitol	Mannitol	Dextrose	Salicin	Biovar
NCIS 1	+	+	+	-	-	-	+	-	**2**
NCIS 2	+	+	+	+	+	+	+	-	**3**
NCIS 3	+	+	+	+	+	+	+	-	**3**
NCIS 4	+	+	+	-	-	-	+	-	**2**
NCIS 5	+	+	+	-	-	-	+	-	**2**
NCIS 6	+	+	+	-	-	-	+	-	**2**
NCIS 7	+	+	+	+	+	+	+	-	**3**
NCIS 8	+	+	+	-	-	-	+	-	**2**
NCIS 9	+	+	+	+	+	+	+	-	**3**
NCIS 10	+	+	+	-	-	-	+	-	**2**
NCIS 11	+	+	+	-	-	-	+	-	**2**
NCIS 12	+	+	+	-	-	-	+	-	**2**
NCIS 13	+	+	+	-	-	-	+	-	**2**
NCIS 14	+	+	+	-	-	-	+	-	**2**
NCIS 15	+	+	+	-	-	-	+	-	**2**
NCIS 16	+	+	+	-	-	-	+	-	**2**
NCIS 17	+	+	+	-	-	-	+	-	**2**
NCIS 18	+	+	+	-	-	-	+	-	**2**
NCIS 19	+	+	+	-	-	-	+	-	**2**
NCIS 20	+	+	+	-	-	-	+	-	**2**

+ Positive reaction (colour of medium changed from green to yellow);

- Negative reaction (colour of the medium not changed). Source: Kinyua *et al.,* (2014).

NCIS: Nakuru County Isolates

3.5 Conclusions and Recommendations

The present study showed that bacterial wilt is prevalent in Nakuru County but differed with the Wards and the altitude. Elevation is an important factor to consider in potato production in respect to bacterial wilt since the study indicated that the disease incidence decreased as altitude increased. The growth stage of bacterial wilt expression is an important lead in seed selection

practices such as positive selection. This should be done when symptomatic expression is apparent in the potato plants to avoid selection of infected plants. Availability of a susceptible host is a major contributing factor to the disease spread as observed in the case of Variety "Shangi' which was the most common variety grown in the region. Unavailability, unaffordability and inaccessibility of clean or certified seed potato also enhances practices such as self-sourcing and use of degenerated seed. The identification of biovar 2 from bitter sweet nighshade and biovar 3 from purslane weed means that farmers need to be sensitized on the importance of weeding them out since they could be alternative hosts of the pathogen.

Promotion of positive seed selection at the right stage in Wards where the disease is prevalent would improve the seed quality and consequently high yields. The study recommends urgent measures to curb bacterial wilt in Nakuru County and especially along the Wards within the Western Mau forest. It would be necessary for the relevant authorities to provide clean seed in the area to avoid further spread of the disease since seed source is a contributing factor to bacterial wilt proliferation in the County, especially in Wards such as Mau Narok, Kuresoi, Bahati, Kamara, Elburgon and Olenguruone. The presence of active government stakeholders such as MOA, ADC and CIP have played an important role in curbing the disease since most farmers were aware of the disease, its symptoms and its means of spread. However, the study indicated poor farmers' practices in curbing the disease and therefore the farmers need to be taught on seed renewal, seed sourcing, crop rotation patterns, etc. Exchange of information on wilt management practices from one Ward to another would be of benefit since the management differs in these sub Counties. More capacity building is necessary to make the farmers aware of how the disease is spread. Control of seed movement would also be a necessary precondition to limit spread of the pathogen to areas of low prevalence such as Keringet and Mauche. Production of clean seed should also be promoted in the sub Counties with low prevalence since the risk of field contamination is comparatively lower as compared to the other sub Counties. Development of cultural practices that are applicable and affordable in the County would contribute in reducing the bacterial wilt spread in the Wards. Identification of biovars in a given geographic place is only one way of classification of *R. solanacearum*. Molecular studies are needed to reveal the diversity of phylotypes and sequevars among strains that exist in Nakuru and other Counties in Kenya.

CHAPTER FOUR

IMPACT OF CROP ROTATION SEQUENCES ON POTATO PRODUCTION IN BACTERIAL WILT-INFESTED FIELDS

4.1 Abstract

The potato industry in Kenya is threatened by bacterial wilt because most production areas are infested with the wilt-causing *Ralstonia solanacearum* and over 50% yield losses have been reported. Continuous cultivation causes soil physical and biological constrains that greatly affect the crop performance and increase proliferation of the bacterium. Rotation with non-host or suppressant plant species could contribute to considerable reduction of bacterial wilt in subsequent potato crops. This study tested the effect of different crop sequences on *Ralstonia solanacearum* population in the soil, wilting incidence and yield of potato. Crops used were spring onion, garden pea, potato, wheat, barley, canola, *Dolichos lablab,* cabbage, sweet potato, desmodium, maize and lucerne. Three season field experiments were conducted at two sites (Egerton University, Njoro and Kenya Agricultural and Livestock Research Organization (KALRO)-Kabete with 17 different crop sequences. Rotations involving brassica and legumes with potato gave a higher emergence percentage of potato compared to the other sequences. There was a significant effect $F(16,119) = 7.063$, $P<0.001$ of the crop rotation sequences on the wilting incidence of potato. Pre-cropping potato with spring onion and barley resulted in a significantly lower wilting incidence compared to all the other crop rotation sequences with a mean of 8.3% across sites. The results showed that Potato-*Dolichos lablab* -potato and cabbage-*Dolichos lablab*-potato had the highest yield with 19.9 and of 19.7 tons/ha in the one crop rotations with potato and pre-crops to potato respectively. A Genotype x Environmental means versus first principal component of the interaction IPCA score showed that the yield due to barley-spring onion, spring onion-barley and wheat-spring onion as pre-crops were more stable in both locations compared to the other cropping sequences. The long season crops used did not have any significant effect on the variables tested. The study indicates that rotations involving spring onion with the locally grown cereals such as barley and wheat can be utilized in curbing bacterial wilt. Rotations involving *Dolichos lablab* and cabbage may also be used to increase the yield of potato in bacterial wilt-infested fields.

4.2 Introduction

Bacterial wilt, caused by *Ralstonia solanacearum,* is reported to be one of the major challenges affecting potato farmers in Kenya (Ateka *et al.,* 2001; Kaguongo *et al.,* 2009; Nyangeri, 2011, Kwambai *et al.,* 2011). Small scale farmers own small land parcels that limit them from implementing crop rotation and therefore practice continuous cultivation of crops in bacterial wilt infested soils. The question is therefore; what kind of cropping systems can be adopted by small scale farmers to reduce bacterial wilt infection in potato crop and thereby strengthen potato production?

Crop rotation as a cultural control method is applied with the objective of achieving maximum benefits due to the contribution of the crops to the soil and the crops effect to pathogen population. The use of different crops in a rotation regime is based on several principles such as use of non-host crops, i.e. with suppressive effect due to their exudates and secondary metabolites, N fixing plants, plants with high residual matter and their adaptability to specific environments. Plants release secondary metabolites in significant amounts at varying stages of growth. Some of the reported metabolites include; Benzoxazinoid-2, 4-Dihydroxy-7-methoxy-2H-1, 4-benzoxazin-3(4H)-one (DIMBOA) which is the most common benzoxazinoid found in wheat and wild barley, among other cereals (Fall and Solomon, 2011). It is reported to cause allelopathy, repel insects and also resist pathogens. They are stored as inactive glucosides in the plant but become active upon tissue disruption. They cause mutagenic effects on pathogens DNA and react with amino acids. Flavonoids are also present in barley in the form of lutonarin, saponarin and isovitexin. Several phytoanticipans in the flavonoids group are reported to inhibit spore germination and the growth of *Xanthomonas oryzae* (Padmavati *et al.,* 1997). UV-absorbing flavonoids in cotton leaf tissues have also been found to be antagonistic to *Xanthomonas campestris* (Edward *et al.,* 2008).

Host exclusion or withdrawal is achieved by growing non-hosts crops in any crop rotation regimes or allowing for fallow period. The duration taken in the crop rotations highly determines the reduction of inoculum in the soil. Short term crop rotations have been shown to affect pathogen populations and disease status in the soil (Lemaga, 2001; Narayanasany, 2013). However research on long-term rotations have shown that crop rotations require more time to

significantly and effectively reduce soil borne pathogens (Larkin *et al.,* 2010; Wright *et al.,* 2015). Rotational crops are also used as cover crops to suppress the disease both in the growing phase and in the decomposition phase by inducing an increase in soil microbial biomass and in soil biological activity. Residue quality is known to indirectly influence the organic matter content and aggregation which impacts the microbial community in the soil. A marked difference has been observed in the levels of carbon in the soil after root and shoots were incorporated into the soil (Ball *et al.,* 2005). Shoots are considered to breakdown rapidly compared to the roots and therefore are a short term source of nitrogen to the subsequent crop. Decomposition of incorporated crop residues also varies depending on the C: N ratio and lignin content of the crop residues. Residues with a low C: N ratio (<25:1) are shown to decompose rapidly thereby creating a suitable substrate for microbial activity (Kriaučiūnienė *et al.,* 2012). Dolichos bean has been found to decompose rapidly with a reduction of at least 25% to 63% of their initial dry weight within the first four weeks compared with other crops (Ibewiro *et al.,* 2000; Ruiz-Vega *et al.,* 2010).

Another factor that influences disease status in the soil as a result of crops grown is the chemotaxis effect of exudates, organic and amino acids from different cover crops or their green manure (Yao and Allen, 2006). Chemotaxis is a factor that promotes the proliferation of pathogens and contributes to the infection rate of the host plant in crop rotation systems. It is reported that *R. solanacearum* strain K60 was attracted both to plant root exudates of tomato which is a host plant and rice, a non-host plant to the pathogen. However tomato root exudates indicated three times stronger attraction compared to rice exudates at protein concentrations of 100ug/ml. This study demonstrated that non-host acids and root exudates are reported to be less attractive to *R. solanacearum* or are repellant (Yao and Allen, 2006). Proliferation of different microbes is also influenced by the different cover crops or green manure and has been observed to vary significantly according to the type of cover crop (Patkowska and Konopiński, 2014). Other factors such as the formation of DNA-containing extracellular traps by non-host plants also play a role in the proliferation of pathogens in the soil (Hawes *et al.,* 2016; Tran *et al.,* 2016).

The efficacy of any crop rotation is determined by the type of soil, soil pH, soil moisture, weather and other abiotic factors and therefore, crop yield and disease incidence may vary from

one location to another and from one season to another (Adebayo and Ekpo, 2006)**.** It is therefore important to consider crops for rotation in any specific environment based on their adaptability. The present study therefore used different crops grown by most farmers in medium to high altitudes areas of Kenya to evaluate their impact on potato yield and bacterial disease when utilized in crop rotations. This was carried out in fields inoculated with *R. solanacearum* to simulate farmers' fields infested with bacterial wilt. The objective of this study was to identify alternative crops besides the potato monoculture that can be used to reduce the effect of bacterial wilt and consequently increase yields.

4.3 Materials and Methods

4.3.1 Site description

Three season field experiments were conducted at two sites: Egerton University, Njoro (Field 7) and Kenya Agricultural and Livestock Research Organization (KALRO), Kabete field stations in 2012 and 2013. The two sites are designated research institutions. The climatic and edaphic characteristics of these locations are presented in Table 4.1.

Table 4. 1: Climatic and edaphic characteristics of the experimental sites

Site	GPS Co-ordinates	Rainfall (mm)	Temp ^{o}C	Soil type	pH
Egerton	2225m asl 01^{o}13'S and 35^{o}30'E	1012	22	Sandy loam Mollic phaozems	5.5-6.0
KALRO	1737m asl 01^{o} 15'S and 36^{o} 41'E	980	23	Clay loam Humic nitisol	4.5-5.5

Source: Jaetzold and Schmidt, (1993); Oloo *et al.,* (2011)

4.3.2 Preparation of inoculum

For purposes of inoculum preparation, the procedures of Kinyua et *al*., (2014) were followed. Tubers symptomatic of bacterial wilt were washed under running water to remove any attached soil. They were immersed in ethanol (70%) for 3 minutes. The tubers were then macerated and immersed in sterile distilled water to obtain a bacterial suspension. The bacterial suspension was

streaked on to semi selective medium (Appendix 7) agar medium and incubated at 28°C for 48 hours. Wild-type bacterial colonies (based on colony morphology) were harvested, suspended in CPG liquid medium (Casamino acids-peptone-glucose) (Appendix 6) and incubated for three days at room temperature. The cultures were suspended in distilled water and adjusted to 10^8 CFU ml^{-1} as described by Yadessa *et al.* (2010).

4.3.3 Inoculation of experimental field

The experimental fields were inoculated with *R. solanacearum* inoculum to simulate the farmers' fields which are infested with the pathogen. Disease infested plots were developed by growing a susceptible potato variety "Tigoni" for a season. Ten millilitres of the inoculum was sprayed to the rhizosphere of each plant at the 30^{th} day after planting according to Ayana *et al.* (2011). All the potato plants were ploughed and incorporated into the soil after more than 50% of the plants showed wilting symptoms.

4.3.4 Crop rotation sequences

There were two crop rotation experiments: in the first one, potato was rotated with short-season crops and in the second case, potato was rotated with long-season crops. The first experiment had 17 crop sequences as the treatments using short season crops. There were sequences where other crops were rotated once with potato while other sequences had two crops planted before the potato crop also termed as pre-crops. Crops used were; spring onion, garden pea, wheat, barley, Canola, *Dolichos lablab* and cabbage (Table 4.2). The treatments were Potato-Cabbage-Potato (1), *Dolichos lablab* -Cabbage-Potato (2), Potato-Canola-Potato (3), Garden Pea-Canola-Potato (4), Potato-*Dolichos lablab* -Potato (5), Cabbage-*Dolichos lablab* -Potato (6), Potato-Garden Pea-Potato (7), Canola-Garden Pea-Potato (8), Potato-Spring Onion-Potato (9), Wheat-Spring Onion-Potato (10), Potato-Wheat-Potato (11), Spring Onion-Wheat-Potato (12), Potato-Barley-Potato (13), Barley-Spring Onion-Potato (14), Spring Onion-Barley-Potato (15), Potato-Potato-Potato (16) also referred to as monoculture in the text, Fallow-Fallow-Potato (17) – in this case the plots were not ploughed or weeded in this treatment throughout the fallow seasons. In the second experiment, the long season crops sequences were as follows; Potato-Fallow-Potato (1), Potato-Sweet Potato-Potato (2), Potato-Desmodium-Potato (3), Potato-Maize-Potato (4), Potato-Lucerne-Potato (5) and Potato-Potato-Potato (6) (Table 4.3)..

Table 4. 2: Description of rate, spacing and sources of seeds used in the study

Crop	Variety	Rate	Spacing	Seed Source
Spring onion	Green bunching	6 kgs /ha seed	20 cm x Drill	Kenya seed
Garden pea	Peas plum	75 kgs/ha seed	50 x 10 cm	Kenya seed
Potato	Tigoni	50kgs/ha seed	75 x 30 cm	CIP
Wheat	Kwale	125kg//ha seed	Broadcast	KALRO-NJORO
Barley	Sabini	50kgs/ha seed	20cm x Drill	EABL-MOLO
Canola	Tower	45kg/ha	30cm x Drill	KALRO-NJORO
Dolichos lablab	Local variety	20kgs/ha	50 x 30 cm	Local market
Cabbage	Copenhagen	30 seedlings per plot	60 x 45 cm	Kenya seed
Desmodium	Silver leaf	5 kgs/ha seed	45cm x Drill	Kenya seed
Lucerne	Local	15kg/ha seed	20cm x Drill	Kenya seed
Maize	H624	25kgs/ha	75cm x 30cm	Kenya seed
Sweetpotato	Local	2 vines per mound	80cm x 30cm	Local

KALRO: Kenya Agricultural Livestock and Research Organization, CIP: International Potato center

Table 4. 3: Sequence of crops in crop rotation experiment

Inoculation stage	Season 1	Season 2	Season 3
Short season crops			
Potato	Potato	Cabbage	Potato
Potato	*Dolichos lablab*	Cabbage	Potato
Potato	Potato	Canola	Potato
Potato	Garden pea	Canola	Potato
Potato	Potato	*Dolichos lablab*	Potato
Potato	Cabbage	*Dolichos lablab*	Potato
Potato	Potato	Garden pea	Potato
Potato	Canola	Garden pea	Potato
Potato	Potato	Spring onion	Potato
Potato	Wheat	Spring onion	Potato
Potato	Potato	Wheat	Potato
Potato	Spring onion	Wheat	Potato
Potato	Potato	Barley	Potato
Potato	Barley	Spring onion	Potato
Potato	Spring onion	Barley	Potato
Potato	Potato	Potato	Potato
Potato	Fallow	Fallow	Potato
Long season crops			
Potato	Potato	Sweetpotato	Potato
Potato	Potato	Maize	Potato
Potato	Potato	Potato	Potato
Potato	Potato	Fallow	Potato
Potato	Potato	Lucerne	Potato
Potato	Potato	Desmodium	Potato

4.3.5 Experimental layout and agronomic practices

The experiments were laid out in a randomized complete block design with four replicates in plots measuring 3m by 3m. For short season experiments, two cropping patterns were considered; rotation of potato with one-crop termed as one crop rotation and use of two different crops before the main potato crop considered as pre-crops to potato. For the long season experiment, crops were rotated with potato only once. The crops were planted in the short rains of 2012, long rains of 2013, and short rains 2013 in succession as indicated in the above treatment sequences. After every harvest, the above ground vegetative biomass was left on the ground and incorporated in the soil using a hand hoe during the planting of the next crop in the next season. The seed potato ("Var. Tigoni") was planted in the last season at a spacing of 75 cm by 30 cm in furrows giving a total of 40 plants per plot. Hand weeding was done at 4 weeks after planting and hilling (earthing up) was done twice at four and eight weeks after planting. Late blight was controlled with Dithane-M45 and Ridomil-MZ 72 sprayed at alternating times at a rate of 5g/10 liters of water when it was necessary. The experimental field was fenced all around and a trench measuring 3ft deep by 3ft in width was dug across the lower slope of the field. A disinfection foot bath was constructed at the entrance of the experimental fields. Sodium hypochlorite (5.25%) at a dilution of 1:10 was used for disinfection at the foot bath and replaced every 24 hours. Every user was expected to disinfect his/her boots and equipment before entering or exiting the fields. After the experiments, the fields were planted with maize for three seasons.

4.3.6. Data collection

(i) Determination of potato emergence: The emergence of potato after planting was calculated as the number of emerged plants expressed as a percentage of the total number of seed potato planted.

(ii) Assessment of bacterial wilt incidence: Assessment of the bacterial wilt incidence started at the onset of wilt symptoms after which counting of wilted plants was done on a weekly basis. Plants that showed either complete or partial wilting were considered wilted and tagged to avoid double counting in subsequent assessments and also to avoid the possibility of missing out those completely killed early in the growth period. Wilt incidence for each treatment was calculated as number of wilted plants expressed as a percentage of the total number of plants emerged (Manmathan and Lapitan, 2013).

(iii) Determination of R. solanacearum in the soil: The population of *R. solanacearum* in the soil was established three weeks after planting in the first season and at 15 weeks after planting of each crop in the subsequent seasons. Bacterial population after the second crop was considered to evaluate the impact of the crop sequences. Four soil samples were randomly picked from each experimental plot at 20 cm soil depth and were mixed thoroughly to make one composite sample. Ten grams of soil from each sample was put in a flask with 30 mls of distilled water. The soil suspension was stirred on a rotary shaker at 150 rpm for 30 minutes. The soil suspension was allowed to settle and 1ml aliquot suspension was drawn out using a sterile pipette tip. This suspension was put in sterile Eppendorf tubes and formed the stock suspension. Serial dilution was carried out upto10^{-3} suspension. An aliquot of 100µl (0.1ml) of the soil suspension was lawn-plated on Semi Selective Medium (SMSA) (Appendix 7) in a petri plate for 10^{-1} and 10^{-3} serial dilutions suspensions. The plates were incubated at 30°C for 48-72 hours. The colonies that showed typical *R. solanacearum* characteristics; fluidal and irregular with a characteristic red or pinkish red centre and whitish periphery, were counted from the 10^{-3} serial dilution suspension (Appendix 8). The number of colonies per ml was calculated using the following formula according to Mehotra and Aggarwal (2003). In this study the volume plated was 0.1 mls and the dilution used was 10^{-3}.

$$Number\ of\ colonies\ per\ gram\ = \frac{CFU\ \times Dilution\ factor}{Volume\ plated}$$

Log transformation of bacterial population data in each of the crop rotations was done for the purpose of analysis of variance; (Lg10) cfu*10000).

*(iv) Determination of potato yield***:** Potato yield data was taken from the last potato crop in the sequences. Harvesting was done once at 110 days after planting. The weight of tubers per sample was recorded and the tubers grouped into three categories based on the diameter of the tubers; ware (>55mm), seed (35-55 mm), and chatt size (<35mm) and the weight was recorded for each plot (Personal communication, Agricultural Development Corporation, 2013).

4.3.7 Data analysis

Data was analyzed for the response variables (emergence (%), wilting incidence weeks after planting (WI WAP %) and yield (tons/ha) using analysis of variance (ANOVA). Post hoc mean separation was done using Tukey HSD whenever there were significant results. Spearman *rho's* correlation was done to examine the relationships between the response variables. IBM SPSS statistic software Version 20 was used for the analysis of this data. To determine the stability of the crop sequences in the two environments, AMMI analysis was done and an IPCA versus Genotype x Environmental means plot was generated to graphically visualize the mean performances and stability of the cropping sequences on the yield of potato (GENSTAT Version 15).

4.4 Results and Discussion

4.4.1 Effect of short season crops rotations on emergence percentage of potato.

Rotations involving brassica and legumes gave higher emergence percentage of potato in the last season compared to the other treatments in both rotation sequences and in both sites (Table 4.4). Cabbage-*Dolichos lablab* and Garden pea-Canola as pre-crops recorded the highest emergence percentage in the subsequent potato crop. This was attributed to the rapid decomposition and mineralization of vegetables and the contribution of *Dolichos lablab* to fertility in the soil as was also observed by Aganga and Tshwenyaye (2003), Sanginga (2003) and Agneessens *et al.* (2014). Rotations involving wheat and spring onion generally gave a lower emergence percentage compared to the brassica-legume rotations. This may be attributed to the allelopathic effect reported in wheat. Wheat contains allelochemicals such as phenolics and alkaloids found in the leaves, roots, and seeds which have been shown to have suppressive effects in the germination of several crop seeds. *In-vivo* and *in-vitro* trials have shown the efficacy of cereals such as barley in suppression of germination in most seedlings such as lettuce, bread wheat, cabbage and alfalfa (Kremer and Ben-Hammouda, 2009). Wheat straw has been known to have a positive allelopathic effect in the reduction of the density and biomass of weeds. It inhibits the growth and yields of other crops such as rice, barley, cotton and soybean (Lam *et al.,* 2012).

4.4.2 Effect of short season crop rotations on bacterial density in the soil

Bacterial population was significantly influenced by the location from the initial inoculation stage to the third season; [F (1, 102) =53.2, P<0.001], [F (1, 102) =12.5, P< 0.001] and [F (1, 102) =236.8, P<0.001], respectively, which is attributed to the different environmental (rainfall, temperature and soil) parameters. A major factor that contributed to the significant effect of the location as a main effect was the soil pH. KALRO site had low soil pH which means the soils were acidic (Table 4.1). There was a decreasing trend of the mean bacterial population of the two sites from the first season to the third season in both crop rotation patterns. The mean wilting incidence (WI) and average bacterial population of *R. solanacearum* of the two sites were higher (21%) in crop sequences involving only one crop in rotation with potato as compared to the sequences involving two pre-crops to potato (13%) (Figure 4.1).

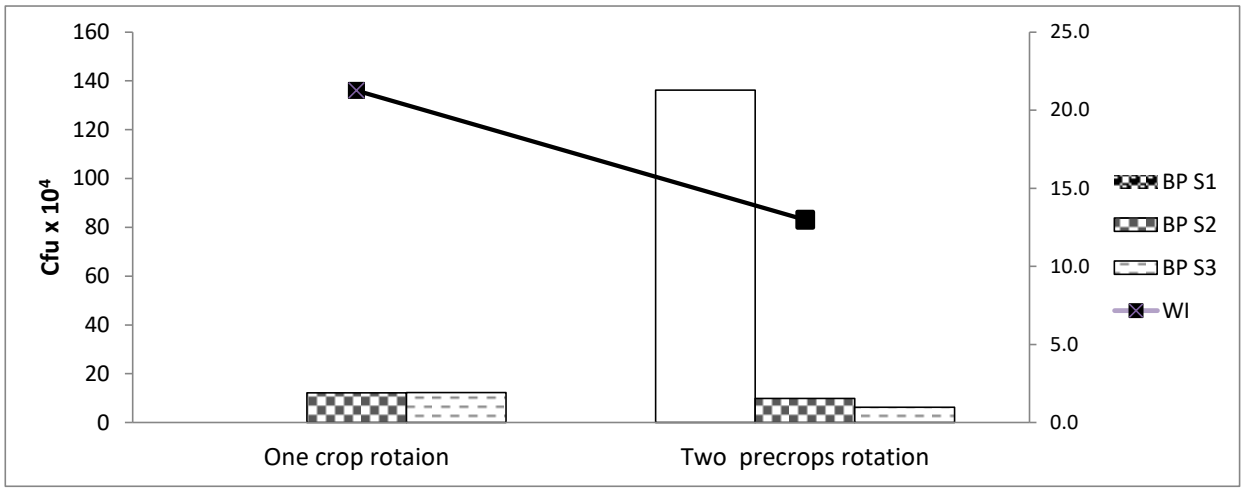

Figure 4. 1: Effect of crops sequences on *R. solanacearum* population and percent wilt incidence of potato in the third season

Values are means of the cropping sequences in each cropping pattern.

BP: Bacteria Population; S: Season; WI: Wilting incidence

The mean yield was also higher in the two pre-crops rotations (19 tons/ha) compared to the one crop rotations with potato (17 tons/ha). The lower wilt incidence due to two seasons of rotation with different crops (pre-crops) may be attributed to the break period which is a physical mechanism in controlling pathogen effect by withdrawal of the host. The absence of the host for two seasons in the two pre-crop rotations resulted to a higher decline in the pathogen density compared to the one crop rotation sequences.

Bacterial population after the second season was considered so as to evaluate the impact of the rotation crops on bacterial population in reference to the initial inoculation stage (Figure 4.2 and 4.3). The pre-crop of wheat-spring resulted to the highest decline of the *R. solanacearum* density in the soil (Figure 4.3). Strongly acidic clay soils are reported to favour the survival of *R. solanacearum* (Sharma, 2004) and this may have contributed significantly to the high wilting index at KALRO site. A combination of the anti-microbial effect of wheat and onion may have contributed to the reduced density of *R. solanacearum* in comparison to the other crop sequences.

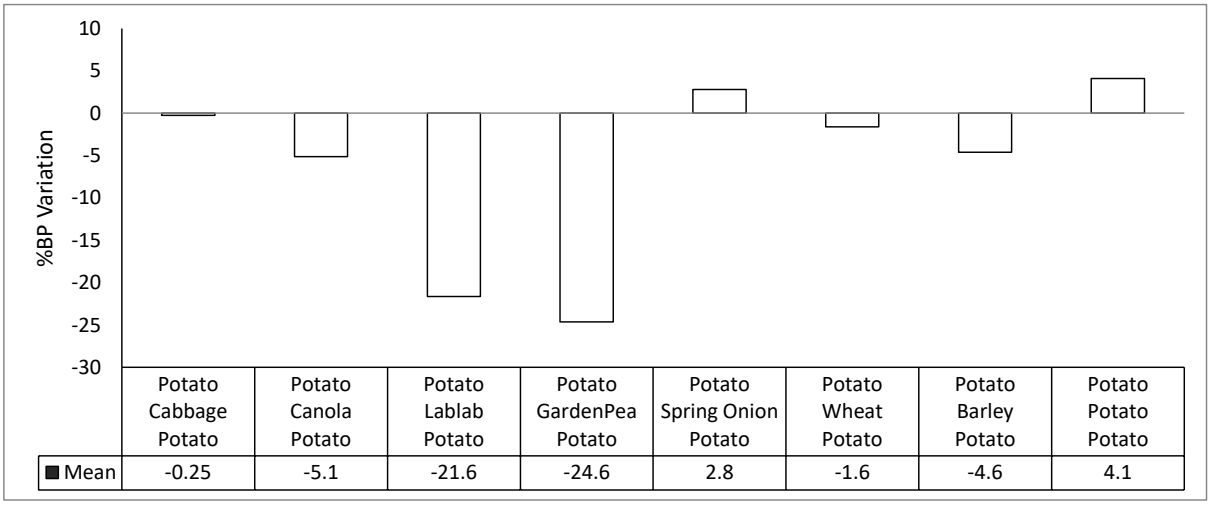

Figure 4. 2: Effect of one crop rotation sequences on percent bacteria population in the soil after second crop.

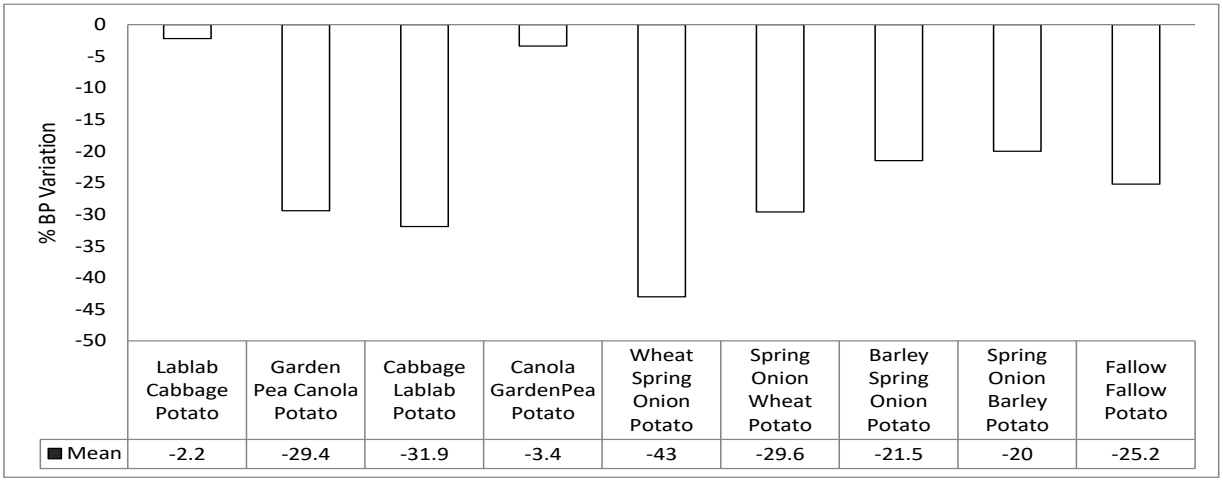

Figure 4. 3: Effect of different pre-crops sequences on percent bacteria population in the soil after the second crop.

BP: % Bacterial Population

Negative or positive figures denote a decline or an increase in the *R. solanacearum* population density respectively in reference to the bacterial population density after inoculation of the experimental field before the first crop was planted.

Onion is reported to have catechol, protocatechuic acid and allicin which have anti-microbial effects (Osbourn, 1996; Tagoe *et al.,* 2011). Flavonoids and benzoxazinoids are found in cereals such as wheat and barley and they have been reported to inhibit growth of several pathogens such as *Xanthomonas oryzae* and *Xanthomonas campestris*, (Padmavati *et al.,* 1997; Edward *et al.,* 2008; Fall and Solomon, 2011).

4.4.3 Effect of short season crop rotations on bacterial wilt incidence in the potato crop

The wilting progress of potato in the last season was observed weekly and it varied significantly with the crop sequences. The progression of wilt in potato-cabbage-potato rotations did not significantly differ from the potato monoculture-no rotation (negative control). Pre-cropping potato with spring onion and barley resulted in a significantly lower wilting incidence than in the other crop rotation sequences with a mean of 8.3% and 2.2% wilt incidence in KALRO and Egerton sites respectively. Spring onion-wheat-potato also had the lowest mean wilting incidence of 10.1% in both sites (Table 4.4). Spring onion has been recommended as a non-host to *R. solanacearum* and suitable for crop rotation with potato (Wang and Lin, 2005). Potato-barley-potato rotation had the least WI in the one-crop rotations with a mean of 12.1% across the two sites. Rotation of potato with cereals such as wheat and barley and spring onion was found to consistently influence the reduction of bacterial population and, consequently reduce, the wilting incidence (Table 4.4).

Cabbage (*Brassica oleraceae* L.) is reported to be a common host plant of bacterial wilt and has also been found to be infected by *R. solanacearum* biovar 2 and 3 (Alvarez *et al.,* 2008; Guidot *et al.,* 2014; Nortj'e, 2015). A study evaluating the effect of different plants on wilt incidence and their infection by *R. solanacearum* biovar 2 and 3 showed *Brassica oleraceae* var. *capitata* is a host to the bacterium whereas spring onion did not show any wilting nor was it infected by the two biovars (Nortj'e, 2015). This study also indicates that barley-spring onion has potential to suppress diseases and concurs with similar results from a previous study showing that barley/clover causes a reduction of fungal diseases in the short term (Larkin *et al.,* 2010). The

effect of barley and wheat in the suppression of disease in the subsequent crop and reduction of the bacteria population may be attributed to root exudates-microbial interaction.

The study also highlights a scientific question whether the order of the crops has an interactive effect on disease or pathogen inoculum. According to this study, pre-crops sequences where spring onion was planted first then cereals resulted to a lower wilting incidence compared to where cereals were planted first then spring onion. Previous studies have shown a positive contribution of canola to reduction in fungal soil-borne diseases (Bednarek *et al.,* 2009; Larkin *et al.,* 2010; Boydston *et al.,* 2011; Bohinc *et al.,* 2012). Research carried out by Ayana and Fininsa (2016) demonstrated that two seasons rotation of growing tomato after bean-maize and maize-bean resulted to 29% wilt incidence reduction. The effect of two season rotations in bacterial wilt has also been demonstrated by Lemaga *et al.* (2001).

4.4.4 Effect of the short season crop rotations on the yield of potato in the third season.

The yield of potato in the third season was significantly lower at KALRO site compared to Egerton site. This is attributed to the acidic soils (Table 4.4) found in KALRO which favour proliferation of *R. solanacearum*. A significant negative correlation (spearman's' rho) of r^2 = -0.419 was observed between yield and the bacterial wilt incidence in potato at 110 days after planting. Potato-*Dolichos lablab* potato and potato-canola-potato had the highest yield with 19.9 and 18.2 tons/ha respectively in the one crop rotations across the two sites. Cabbage-*Dolichos lablab* and *Dolichos lablab* -Cabbage as pre-crops to potato also resulted to the highest yield of 19.7 and 19.0 tons/ha across the two sites respectively. As shown in Table 4.4, rotation of potato with *Dolichos lablab* and canola yielded the highest in Egerton and KALRO sites respectively in the one-crop rotations with potato.

Dolichos lablab is able to transport minerals from the depths of the soil to make it available to the plants due to its deep tap root and it's active role in N fixation in the soil due to the presence of N fixing bacteria (Aganga and Tshwenyaye 2003; Sanginga 2003). Legumes are known to form symbiotic relationships with soil borne rhizobia known as plant growth promoting rhizobacteria (PGPR). These plant growth promoting bacteria produce plant growth regulators, are involved in symbiotic N fixation and solubilize minerals such as phosphorus, among other beneficial mechanisms that are important to crops (Cooper, 2008; Montano, *et al.,* 2014).

The presence of canola and cabbage in the brassica-legume patterns in the highest yielding pre-crop crop sequences may also have played a role in the yields increase due to the short duration taken by most vegetables for mineralization after incorporation into the soil surface (Agneessens *et al.,* 2014). A combined effect of the legumes fixing nitrogen and vegetables decomposing in the short term is an aspect that would have positively impacted yield of potato in the third season. A study done by Nyangeri (2011) indicated that a potato-cabbage-potato recorded significantly marketable yields of potato compared to other crops such as maize and beans. Another research evaluating the rotation effects of canola, barley, and green beans to potato yield showed that canola resulted in significantly higher yields of potato compared to the other crops (Larkin *et al.,* 2010). This study concurs with these previous results indicating the potential of brassicas in rotation with potato in increasing tuber yield. Residues with a high C: N ratio, high lignin and polyphenols content are known to immobilize inorganic N, resulting to reduced microbial activity and, therefore, reduced yields (Kumar and Gor, 1999). This may have contributed generally to the lower yields in the wheat and barley rotations with potato when compared to the rotations with legumes and brassicas.

Table 4. 4: Results of different crop rotation sequences on emergence, yield and percent wilting incidence of potato in third season

Treatments	Emergence (%)		Yield (Tons/ha)		WI 5 WAP		WI 10 WAP		WI 15 WAP	
	Eger	KALRO	Eger	KALRO	Eger	KALRO	Eger	KALRO	Eger	KALRO
Spring Onion Barley Potato	86.3±8.2	63.1±16.7	29.6±4.5ab	7.8±2.0a	0.0±0.0a	0.0±0.0	0.7±1.3a	0.8±1.7a	2.2±2.9a	14.2±4.8a
Spring Onion Wheat Potato	80.6±11.3	65±6.1	27.5±5.1ab	7.8±1.6a	2.1±2.7a	1.0±0.0	4.0±4.6a	1.1±2.2a	6.1±6.0ab	14.1±8.6a
Canola Garden Pea Potato	79.4±12.5	65.6±10.8	30.9±2.6a	7.1±0.25ab	1.5±3.0a	0.0±0.0	3.6±4.2a	0.8±1.7a	5.3±2.2ab	15.0±11.5a
Barley Spring Onion Potato	80.6±6.9	72.5±10.2	28.9±2.3ab	7.5±0.9ab	0.7±1.5a	0.0±0.0	3.0±2.4a	0.8±2.5a	6.1±4.1ab	16.7±3.6a
Lablab Cabbage Potato	84.4±4.5	70.6±8.7	31.4±3.1a	6.5±1.4ab	6.0±6.9a	0.0±0.0	6.5±10.5a	0.8±2.2a	11.2±10.1ab	13.4±3.7a
Potato Barley Potato	84.4±5.5	61.8±10.1	32.0±4.3a	4.3±2.4ab	2.9±4.1a	0.0±0.0	5.6±4.7a	0.0±0.0a	6.6±3.5ab	18.5±8.1a
Potato Lablab Potato	83.3±7.5	69.4±14.2	33.2±3.6a	6.7±2.3ab	3.6±4.5a	0.0±1.6	5.7±7.7a	0.8±1.6a	8.7±7.3ab	17.1±12.0a
Potato Wheat Potato	83.3±9.2	61.2±10.9	25.8±4.7ab	5.4±3.8ab	6.6±4.7ab	0.0±0.0	10.8±9.1a	4.3±6.3ab	13.9±5.8ab	12.8±5.4a
Cabbage Lablab Potato	85.6±4.3	72.5±8.9	33.0±5.6a	6.5±0.5ab	9.7±4.3ab	0.0±0.0	11.7±3.9a	0.8±1.6a	13.1±4.7ab	17.1±12.0a
Potato Garden Pea Potato	80.0±16.2	60.6±8.8	28.7±2.7ab	6.8±1.1ab	4.7±2.8a	0.9±1.8	4.7±2.8a	0.9±1.8a	12.0±7.0ab	17.6±11.6a
Potato Spring Onion Potato	77.5±11.9	63.8±13.6	29.4±2.3ab	4.8±3.2ab	3.1±2.5ab	0.0±0.0	5.6±2.8a	1.9±2.3a	8.3±4.2ab	21.9±11.8a
Wheat Spring Onion Potato	71.9±4.3	69.4±9.4	29.2±2.9ab	6.7±1.5ab	6.4±6.1ab	0.0±0.0	9.6±7.3a	2.0±4.0a	16.5±13.1ab	15.0±6.0a
Fallow Fallow Potato	70.6±12.3	73.1±14.8	28.5±4.2ab	8.3±2.2a	8.3±9.0ab	0.8±1.5	8.3±9.0a	0.8±1.5a	15.1±9.9ab	16.9±2.2a
Garden Pea Canola Potato	79.4±8.2	80.0±9.1	26.7±2..4ab	8.9±1.2a	6.9±5.4ab	0.0±0.0	10.0±8.1a	1.4±1.6a	10.6±7.9ab	15.9±5.3a
Potato Canola Potato	80.6±7.7	78.8±4.8	28.7±3.2ab	7.8±1.2a	6.0±7.1a	0.0±0.1	11.4±9.2a	2.4±1.6a	13.8±9.2ab	24.3±9.3a
Potato Cabbage Potato	78.1±8.3	77.5±6.1	26.4±6.3ab	7.6±1.2ab	11.8±3.3ab	0.8±1.6	19.5±7.9ab	2.4±2.9a	23.4±10.4bc	24.5±12.2ab
Potato Potato Potato	79.4±8.3	70.6±12.9	20.7±3.1b	2.7±2.3b	22.7±15.8b	0.8±1.6	36.5±9.9b	10.5±4.8b	39.4±10.4c	45.5±2.4b
Mean	**80.4**	**69.2**	**28.8**	**6.6**	**6**	**0.3**	**9.9**	**1.9**	**12.5**	**18.7**
P values	**0.568**	**0.242**	**0.01**	**<0.01**	**<0.01**	**0.19**	**<0.001**	**<0.001**	**<0.001**	**<0.001**

Values are means of data collected in the last potato season. Values with the same letters within a column are not significantly different according to Tukey's HSD mean separation. The ± refer to Standard deviation

Eger: Egerton, KALRO: Kenya Agricultural Livestock and Research Organization, WAP: Weeks after Planting, WI: Wilting Incidence.

4.4.5 Stability of the crop sequences in the different environments

An additive main effects and multiplicative interaction (AMMI) model which combined the analysis of variance for the crop sequences and location main effects with the principal component analysis (PCA) of the crop sequences-environment interaction was performed on yield data of the potato (Figure 4.4). The yield of potato after barley-spring onion (CR 14), spring onion-barley (CR 15) and wheat-spring onion (CR 10) as pre-crops was more stable in both locations compared to the other cropping sequences. The most suitable cropping sequence with respect to KALRO was potato-wheat-potato while Cabbage-*Dolichos lablab-potato* (CR6), potato-*Dolichos lablab-potato (CR 5)* and potato-barley-potato (CR 13) were more suitable at Egerton site. The effectiveness of crop rotation is highly dependent on the prevailing weather characteristics of any environment, the soil environment which comprises of the physical and biochemical properties of soil and the adaptability of crops being used in the rotations (Ding *et al.,* 2008). This study demonstrated the importance of determining a crop rotation regime that is well adapted to a specific environment for improved yields and reduced effect of bacterial wilt. The concept of environment specific system has also been demonstrated by several authors such as Mrabet (2011) and Seremesic *et al.* (2013).

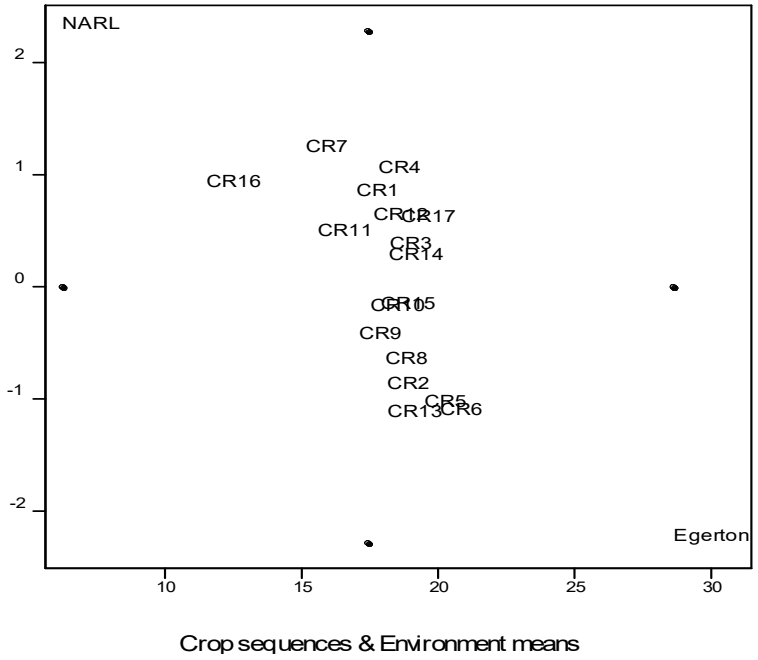

Figure 4. 4: Additive main effects and multiplicative interaction model for potato yield (t/ha) showing the genotype and environment means (X axis) against their respective principal component analysis scores (Y axis)

CR: Crop rotation sequences, NARL (KALRO)

4.5 Effect of long season crops on the performance of potato in bacterial wilt infested soils

A comparison of all the crop sequences to the potato monocultures using the LSD tests did not indicate any significant differences in all the response variables. Location was consistently significant for all the response variables in the study; such as yield, % ware, % seed, and % chatts; [F (1, 84) =30.4, P<0.001], [F (1, 84) =185.3, P<0.001], [F (1, 84) =104.1, P<0.001], [F (1, 84) =22.6, P<0.001] respectively. Crop rotation of potato with *Desmodium intortum* (Mill.) recorded a lower wilt incidence and higher yield in both sites compared to all the other crop rotation sequences (Figure 4.5). Potato-desmodium-potato gave the highest percentage of ware size tubers followed by rotation with maize in KALRO site while potato-desmodium-potato gave the highest percentage of ware size tubers in Egerton site followed by potato-sweet potato-potato. *Desmodium intortum* is reported to be immune to natural and artificial infection by *R. solanacearum* (Smith, 1939). Another study demonstrated that maize reduced bacterial wilt in a maize potato intercrop (Autrique and Potts, 1987). According to this study, maize also resulted to a lower wilting incidence in potato compared to sweet potato, fallow, lucerne and monoculture. Maize is reported to be a non-host to *R. solanacearum and* encourages growth of antagonistic bacteria known as *Pseudomonas cepacia* (Elphinstone and Aley, 1993).

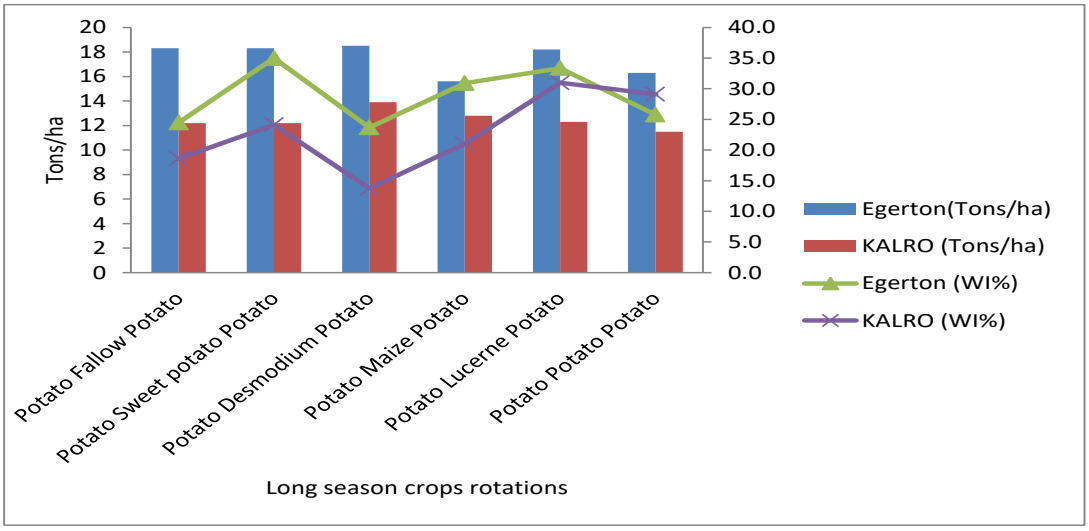

Figure 4. 5: Effect of selected long season crops in rotation with potato on the yield and wilting incidence of potato

4.6 Conclusions and recommendations

The study indicates that Canola can be used in rotations with *Dolichos lablab* with an objective of increasing the emergence percentage of potato. The study also indicated that two successive crops planted as pre-crops to potato are not adequate to destroy all the *R. solanacearum* population or eradicate the disease in the soil especially so if the inoculum is high. Pre-crops of wheat-spring onion and spring onion-barley were effective in reducing *R. solanacearum* density in the soil and wilting incidence compared to all the other crop rotation sequences hence can be recommended to farmers. Pre-cropping cabbage-*Dolichos lablab* and *Dolichos lablab* -cabbage to potato can also be utilized in achieving better yields in bacterial wilt infested soils. Short term evaluation of crop rotations is an important predictor of crops that can be used to suppress bacterial wilt effect in farmers' fields. In conclusion, pre-crops of wheat-spring onion and spring onion-barley are therefore recommended to farmers with bacterial wilt infested fields since they had low IPCA scores with higher yields therefore more stable in both environments. Further studies are recommended to evaluate if the pattern of different crops sequences in a crop rotation regime has an effect on bacterial wilt and pathogen population. According to the study, one crop rotation with long season crops is not effective in reducing bacterial wilt. To establish the effect of the long season crops on bacterial wilt and yield, the experiments can be carried out for a longer period of time.

CHAPTER FIVE

EFFECT OF ORGANIC, INORGANIC SOIL AMENDMENTS AND SEED POTATO TREATEMNT WITH CLEANSTART ON BACTERIAL WILT AND YIELD OF POTATOES (*Solanum tuberosum*)

5.1 Abstract

Bacterial wilt contributes 30 - 70% loss of yield in potato producing regions of Kenya. Infertile soils associated with continuous cultivation of this crop have contributed to the build-up of bacterial wilt pathogen (*Ralstonia solanacearum*). This study evaluated the effects of local and commercial organic and inorganic soil amendments and seed potato treatment on bacterial wilt and yield of potato (*Solanum tuberosum* L.). Field experiments were laid out in a Randomized Complete Block Design (RCBD) with four replications for two seasons at Kenya Agriculture and Livestock Research Organization (KALRO)-Kabete and at Egerton University, Njoro, Kenya. Black majik + NPK, cow manure, Takataka compost and neemgold significantly reduced the wilting incidence at Egerton site compared to the control [$F(7,128) = 2.287$, $P<0.05$]. Seed potato treatment with Cleanstart did not contribute positively to the yield of the potatoes and disease incidence. Pearson's product moment correlation coefficient (r) study showed strong linear relationships between the soil biochemical variables analyzed; total nitrogen, total organic carbon, available phosphorus, pH, yield and wilting incidence. The results of the regression indicated the four predictors (TN, TOC, P, pH) explained 87.2% of the variance [$R^2 = 0.872$, $F(4, 63) = 108.3$, $p \leq .001$] in yield and 68% of the wilting incidence [$R^2 = 0.68$, $F(4, 63) = 34.39$, $P < .001$]. The study indicates that reinforcing inorganic fertilizers with organic amendments is essential in the short term realization of high yields and improving of soil health. Soil pH is an important factor to consider in potato production in respect to yield and bacterial wilt.

5.2 Introduction

Bacterial wilt is influenced by the soil composition since soil forms its habitat and source of nutrition. The soil flora and fauna are important components in the soil health and sustenance of crop production. They are factors in the biological transformations of the soil and are sources and sinks of important nutrient minerals. Incorporation of organic amendments improves the soil physical and chemical environments by increasing the soil organic matter, improving the microbial community and thereby improving the soil health. Organic soil amendments such as farmyard manure and neem cake have been shown to contribute significantly to an increase in the soil carbon compared to inorganic fertilizers (a mixture of urea, Single Super Phosphate (SSP) and muriate of potash (Nakhro and Dkhar, 2010). Organic carbon added to the soil has been found to select for specific microorganisms where bacteria feed on easily available carbon compared to fungi which survive on more complex carbon compounds (Laczano and Domi'nguez, 2011). Organic amendments have been reported to improve soil health by suppressing potato bacterial wilt causing pathogen, *Ralstonia solanacearum,* thereby inhibiting its infection, consequently leading to increased yields of potato. This suppression is attributed to the high microbial activity and improved physical and biochemical characteristics of the soil resulting from organic manure application (Yadessa *et al.,* 2010). A study by Messiha *et al.* (2007) reported a reduction of bacterial wilt by inorganic fertilizers under specific soils. NPK-amendment to Egyptian sandy soil in that study was found to reduce potato wilt severity by almost 100%. The study revealed that bacterial suppression by the organic and inorganic manures varied with the soil types. Not all organic amendments are reported to suppress bacterial wilt. Studies have showed better responses of some organic soil amendments compared to others on the suppression of *R. solanacearum*. Coffee pulps and pork manures are reported to have insignificant effect on suppression of bacterial wilt (Islam and Koki, 2004). Further studies have showed that combinations of organic and inorganic manures are important in achieving high potato yields (Lemaga *et al.,* 2001).

Use of organic fertilizers is a better way to improve the soil health and has been shown to have long term impact in the soil chemical and physical characteristics (Lemaga *et al.,* 2001; Hopkins and Stark, 2003). This increases the soil organic matter thereby enhancing the productivity of crops. Integration of organic amendments to the soil contributes partially in impacting the levels

of organic matter in the soil since there are other fractions of plant roots and residues, living and dead organisms. Once the organic material is in the soil, decomposition takes place, however, at different rates based on the initial quality of the organic amendment (Mohanty *et al.,* 2013). Most of the nutrients are in organic form before decomposition takes place and therefore require being in inorganic form for availability to the plants (Brandy and Weill, 2002).

Soil organic matter is usuall in three pools; active pool, slow and passive pool. The active pool comprises of recent organic matter undergoing mineralization. Inorganic nutrients resulting from mineralization undergo several processes such as immobilization by the soil microbial biomass to organic nutrients being made available for the synthesis of cells and tissues. Organic carbon is a source of energy for the microbial processes. There are also losses of Carbon (C) and Nitrogen (N) due to respiration during decomposition and other losses of soluble inorganic nutrients through leaching (Mohanty *et al.,* 2013). The effect of different soil amendments is apparent at the early stage of decomposition since the rate of decomposition depends on the initial quality such as C: N ratio that alters the nitrogen release and availability. It also determines the availability of sufficient N for soil microbial biomass synthesis and C as an energy source (Mohanty *et al.,* 2013). After immobilization and associated losses occur, the remaining soluble inorganic nutrients (net mineralization) are available for the plant uptake. It is apparent that only a fraction of the total organic carbon, total nitrogen, and phosphorus incorporated into the soil is available to the plants. The presence of these elements in the soil has an significant effect on soil microbial community. Addition of vermicompost and rabbit manure significantly increased bacterial biomass with a marked increase of gram negative bacteria compared to conventional fertilization with inorganic fertilizers such as NPK (Laczano and Domi'nguez, 2011). This indicates that increasing organic carbon with the intent of improving soil health does not necessarily destroy pathogens since this author realised an increase in gram negative bacteria, but also other dynamics such as soil fertility and plant vigour are involved.

Soil pH plays a major role in the availability of nutrients and survival of various microbial communities (Mc Cauley *et al.,* 2009). Low pH and organic matter are reported to provide a favourable soil environment for multiplication of *R. solanacearum* (Ramesh and Bandyopadhyay, 1993; Janse, 1996). Research has shown that the soil physical and bio chemical characteristics for specific soil types have a significant effect on the disease progress, pathogen

survival and soil fertility and therefore the need for evaluation of different amendments on specific production areas. Evaluating the impact of selected organic and inorganic fertilizers on disease and productivity of potato on bacterial wilt infested soils is valuable to farmers especially those who are limited in resources and may not have extra parcels of land for alternative tillage practices like fallow or crop rotations.

5.3 Materials and Methods

5.3.1 Site description

Two season field experiments were conducted at two sites: Egerton University, Njoro (Field 7) and Kenya Agricultural and Livestock Research Organization (KALRO), Kabete in the years 2012 and 2013. The endaphic characteristics are as indicated in Table 4.1 above.

5.3.2 Inoculation of experimental field

The experimental fields were inoculated with *R. solanacearum* according to a method by Kinyua *et al.* (2014). Tubers symptomatic of bacterial wilt were washed under running water to remove any attached soil. They were immersed in ethanol (70%) for 3 minutes. The tubers were then macerated and immersed in sterile distilled water to obtain a bacterial suspension. The bacterial suspension was streaked on TZC agar medium at 28°C for 48 hours. Virulent bacterial colonies (based on colony morphology) were harvested and suspended in Casamino acids, peptone and glucose (CPG) liquid medium and grown for three days at room temperature. Bacterial pellets were suspended in distilled water and adjusted to 10^8 CFU ml^{-1}. Disease infested plots were developed by growing a susceptible potato variety "Tigoni" for a season. Ten milliliters of the inoculum was sprayed to the rhizosphere of each plant at the 30^{th} day after planting (Ayana *et al.,* 2011). All the potato plants were ploughed and incorporated into the soil after more than 50% of the plants showed wilting symptoms.

5.3.3 Soil amendments and seed potato treatment used in the study

The experiment had two factors: Seed potato treatment and soil amendment. Seed potato treatment involved two levels: with Cleanstart [(a pack of four biological products with Rootgard which is a fungicide, Phosgard, Humax which is 80% Humic acid and natural wet (anti stress wetting agent containing saponins from Yucca-10%)] and without Cleanstart. The soil

amendment was in 8 levels namely; cow manure, neemgold (Neem organic fertilizer from Neem kernel cake) and Takataka compost (Composted waste from vegetable and fruits markets) which were applied at 90 kg/ha N and 45 kg/ha N, NPK + black majik. A positive control of commercial NPK and a negative control of no amendment were included. Cleanstart, neemgold, black majik and Takataka compost were sourced from International Potato Centre (CIP). Cow manure (droppings + straw) was piled for three months and mechanical turning took place every three weeks in the first two months to allow for sufficient oxygen flow. Treatments were applied at a rate of 90 kg/ha N except for Takataka compost and Neem gold which were also applied at half rate, i.e 45kg N/ha (Table 5.1). The amount applied per plot was determined from the composition of each amendment as indicated in Appendix 11 and 12. Seed treatment and soil amendments were combined at all possible levels to make 16 treatments.

Table 5. 1: Description of soil amendments and potato seed treatment used in the study

Factor	Levels	Description
Seed treatment	With Cleanstart	Cleanstart is a pack of four biological products with Rootgard-Fungicide: Phosgard (4:25:15), Humax (80% Humic acid: Natural wet (Anti stress wetting agent containing saponins from Yucca-10%).
	Without Cleanstart	
Nutritional soil amendments	Neemgold	Neem organic fertilizer from Neem kernel cake,
	½ rate of Neemgold	45kg N/ha
	Takataka compost	Composted waste from vegetable and fruits markets.
	½ rate of Takataka compost	45kg N/ha
	NPK + black majik	Black magic Potassium 10%), Humic acid (70%), pH 9, CEC-450, Carbon content (50%-60%)
	Cow manure	Composted
	NPK (positive control)	Commercial inorganic fertilizer (17:17:17)
	No amendment (Negative control)	

5.3.4 Experimental layout and agronomic practices

The experiment was laid out in RCBD design with two levels of seed treatment, eight levels of soil amendment with four replicates in plots of 3m by 3m. Furrows were made and the treatments were applied during planting of the potato tubers (Var. "Tigoni") according to the rates described in Table 5.1. Seed treatment was done by dipping the tubers in a solution of 14mls Cleanstart in 30 litres of water for 30 minutes. Treatments were placed in the furrows before planting the seed potatoes. The seed potato was planted at a spacing of 75 cm by 30 cm giving a total of 40 plants per plot. Weeding was done at the 4^{th} week after planting and ridging (earthing up) was done twice at four and eight weeks after planting. Late blight was controlled with Dithane-M45 and Ridomil-MZ 72 sprayed at alternating times at a rate of 5g/10 liters of water when it was necessary. The experiments were carried out in the short rain season (September to December, 2012) and long rain season (March to June, 2013) in the two sites.

5.3.5 Data collection

5.3.5.1 Determination of wilting incidence: Assessment was done on all plants that emerged in the plot. Counts of wilted plants were done at two weeks from the onset of the disease. Plants that showed either complete or partial wilting were considered wilted and tagged to avoid double counting in subsequent assessments and also to avoid the possibility of missing out those completely killed early in the growth period. Wilt incidence for each treatment was calculated using the formula below by Mehotra and Aggarwal (2003).

$$Bacterial\ wilt\ incidence = \frac{Number\ of\ plants\ with\ symptoms}{Total\ number\ of\ plants\ assessed}\ x100$$

5.3.5.2 Determination of the soil macro nutrients*:* Samples from four random points were collected using augers to 20cm depth and bulked for each plot in each site in each season and analysed for total organic carbon, total nitrogen, pH and phosphorus (Appendix 10). The samples were composited for similar treatments in each replication, air-dried and sieved using 2 mm diameter sieve.

Determination of total organic carbon: This was done as outlined by Nelson and Sommers (1982). Total organic carbon (C) was determined by wet oxidation with acidified potassium

dichromate ($K_2Cr_2O_7$) followed by titration of excess potassium dichromate with 0.2 M ferrous ammonium sulphate. A quantity of 0.5 g of ≤ 0.3 mm (60 mesh) soil was placed in a block digester tube and 5 ml of potassium dichromate solution and 7.5 ml of concentrated sulphuric acid were added to the tube and to two reagent blanks. The block digester was placed in a preheated block at 145°C – 155°C and kept at that temperature for 30 minutes. After this period, the digester was cooled and the digests transferred into 100-mL flasks. Then, 0.3 ml of Ferroin indicator was added, and the contents mixed thoroughly. The reagent blanks and the digests were titrated with 0.2 M ferrous ammonium sulphate solution to a brown endpoint.

$$\text{Organic Carbon} = \frac{\text{T X 0.2 x 0.3}}{\text{Sample weight}} \text{ x 100}$$

Where: T = Titre volume (difference between reagent blank and sample solution)

Determination of total N: The determination of total N was done according to the Kjeldal procedure based on Okalebo *et al*. (2002). A distillation and titration procedure was used whereby ammonia liberated from solution by steam distillation was collected in excess boric acid with an indicator. An aliquot (10 ml for soil) of sample solution was transferred to Markham nitrogen still and 10 ml of 40% NaOH added. This was immediately steam distilled into 5 ml of 1 % boric acid containing 4 drops of the mixed indicator (bromocresol green; methyl red and thymol blue mixture). Distillation was continued for 2 minutes from the time the indicator turned green, after which the distillate was removed and titrated with N/140 HCl for soil samples. The end point was reached and the micro-burette reading made when the indicator changed from green through grey to a definite pink. A reagent blank determination was run by distilling and titrating reagent blanks as above. The quantities of N/140 required for the blank were subtracted from the micro-burette readings for plant and soil samples, giving a corrected volume of N/140 HCl. The %N in the soil samples was calculated according to this formula:

$$\%\, N \text{ in soil sample} = \frac{\text{Corrected ml of } \frac{\text{N}}{140} \text{ HCL x 0.1}}{\text{Weight of sample}}$$

Determination of P (ppm): Extractable P was determined as described by Olsen *et al*., (1954). An Olsen's extracting solution containing 0.5 M sodium bicarbonate at pH 8.5 was used to extract P from the soil. The P content of the extract was then determined colorimetrically. Sodium bicarbonate (420 grams) was dissolved in distilled water to a final volume of 10 litres. It was

placed in a mechanical electric mixer to dissolve the sodium bicarbonate. The pH was adjusted to 8.5 by addition of 50% sodium hydroxide. Soil samples weighing 2.5 g were air dried and added into 250-mL polythene shaking bottles. Fifty mililitres of Olsen's extracting solution was added to each bottle. With a firm stopper in place, these were shaken for 30 minutes at 200 rpm on a mechanical-electric shaker. The suspension was then filtered through Whatman paper No 42. For colorimetric measurements, 10 ml of each standard solution, 10 ml of the filtrate and 2 reagent blanks were pipetted into 50-ml volumetric flasks. Five mls of 0.8 M boric acid and 10 ml of the ascorbic acid reagent were then added and each flask was filled to 50 ml with distilled water and shaken. After 1 hour, the absorbance of the solutions was measured at a wavelength setting of 880 nm. From the absorbance of the standards, a standard phosphorus curve was drawn which was used to estimate the concentration of phosphorus in the filtrates. The reagent blank P concentration was subtracted from the sample P concentration to obtain a corrected value. P in the soil (ppm) = corrected P in the solution (ppm) x 100.

Determination of soil pH: Ten grams of soil were placed in a 60-mL beaker and 25 ml of distilled water was added. The mixture was stirred for 10 minutes and allowed to stand for 30 minutes and then stirred again for 2 minutes. The pH reading was done using a pH meter. Before measuring the soil pH, the pH meter was calibrated using pH 4 and pH 7 buffer solutions. The soils were analyzed at the beginning of the experiments and at the end of each season and compared.

5.3.5.3 Assessment of potato yield: Tuber yield per plot was determined by weighing the total harvested tubers at 110 days after planting and recorded. The number of ware size potatoes (>55mm), seed (35-55 mm), and chatt size (<35mm) per plot was counted and calculated as a percentage of the total tubers harvested per plot.

5.3.6 Data analysis

The data was analyzed using the SPSS software package. Analysis of variance was conducted to determine the effect of the treatments on the yield and wilting incidence of potato. Tukey HSD was used to separate the means whenever there was a significant effect. LSD pair wise comparison of treatment was done to compare the treatments with the controls when it was

necessary. Pearson's product moment correlation coefficient (r) was used to examine the relationships between the variables and their relevance to potato productivity

5.4 Results and Discussions

5.4.1 Influence of site on the yield and wilt incidence of potatoes grown in bacterial wilt infested soils

Analysis of variance showed significant differences in yield due to site. Wilting incidence was significantly different $F(1,255) = 639.2$, $P<0.001$, from one site to another. Among the factors that contributed to the varying wilting incidence observed was the total rainfall experienced during the experimental period and the soil pH. The higher the total rainfall recorded and the lower the pH, the more wilting incidence (%) was observed and consequently lower yields. The production period during the short rains (2012) recorded total rainfall of 466mm and 991mm in Egerton and KALRO site respectively according to data from meteorological departments of the respective institutions. The long rain season in KALRO was characterized by heavy rainfall compared to the short rains. This was especially observed in the first two months after planting of the potato crop at KALRO. Total amount of rainfall in Egerton during the production period in the long rains was slightly higher (478mm) compared to the short rains (465mm). Rainfall highly influences the amount of moisture in relation to other soil physiochemical properties which influences bacterial wilt proliferation as also observed by Hayward (1991). The mean temperatures within the growing seasons were 23^{o}C and 20^{o}C in the short rain seasons and 18.5^{o}C and 18^{o}C in the long rains in KALRO and Egerton site respectively. High rainfall and strongly acidic clay soils created a favourable environment for bacterial wilt which significantly caused a high wilting index in KALRO. Strongly acidic soils (<5) are reported to favour proliferation of *R. solanacearum* and also reduce the activity and numbers of beneficial microorganisms in the soil (Sharma, 2004; Mc Cauley *et al.,* 2009).

High bacterial wilt incidence and the low pH observed in KALRO site were considered the causal factors of the low yields (Figure 5.2 and Table 5.2). A negative correlation (spearman's' rho) of r^2 =0.780 at P< 0.01 was observed between yield and the bacterial wilt incidence. The optimum pH of potato production is 5.5- 6.5 according to Tantowijoyo and Elske van de Fliert (2006) and it increases availability of most micro and macro nutrients in plants (Mc Cauley *et*

al., 2009). This factor may have contributed significantly to the high yields in Egerton site which had a pH range of 5.7 to 6.3 compared to KALRO (Figure 5.1 and Table 5.2), The low yield in the KALRO is attributed to the high incidence of bacterial wilt reported in this site.

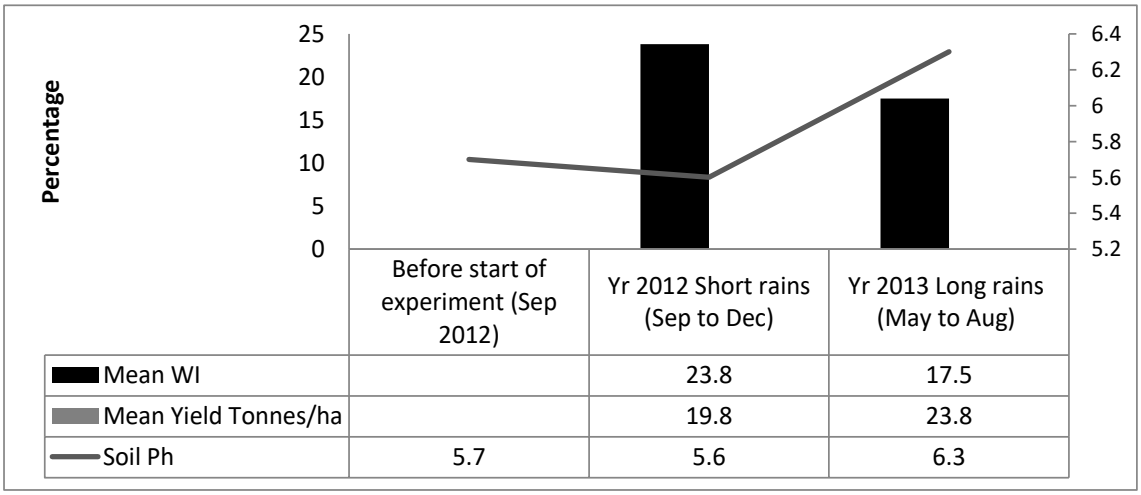

Figure 5. 1: Mean yield and bacterial wilt incidence of potato in the short and long seasons at Egerton site

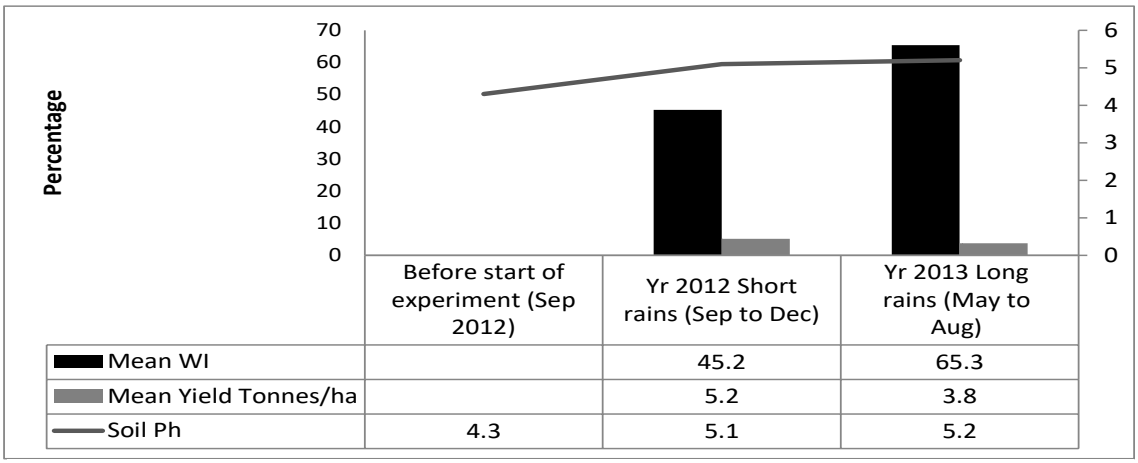

Figure 5. 2: Mean yield and bacterial wilt incidence of potato in the short and long seasons at KALRO site. WI: Wilting incidence

Table 5. 2: Chemical characteristics of the soil in the experimental sites before the study

Site	pH	Total N%	Total OC (%)	P (ppm)
Site 1(Egerton)	5.65	0.33	3.27	50
Site 2 (KALRO)	4.33	0.21	2.03	60

N: Nitrogen, OC: Organic Carbon, P: Phosphorus.

5.4.2 Effect of s oil amendments on yield of potatoes

The analysis indicated significant effects on wilting incidence due to the soil amendments. The highest potato yields were attained with treatments NPK, NPK + Black majik and cow manure in both sites (Table 5.3).

Table 5. 3: Effect of soil amendments on bacterial wilting incidence and yield of potatoes

Treatments	Yield (Tons/ha)		Wilting Incidence	
	Egerton	**NARL**	**Egerton**	**NARL**
Black majik +NPK	22.0±8.7	6.0±2.8	14.5±12.4a	56.0±28.4
Cattle manure	23.2±6.5	4.5±2.3	21.5±17.9ab	49.0±25.8
Neem gold ½	20.0±8.9	3.4±2.1	20.1±15.0ab	52.2±27.3
Neemgold	19.3±5.9	4.4±2.8	15.3±13.8a	57.7±25.0
Takataka compost ½	21.2±7.7	3.9±2.3	24.0±16.4ab	56.3±21.6
Takataka compost	21.1±5.6	4.3±2.9	16.1±12.3a	55.4±21.8
NPK (Positive control)	26.4±5.8	6.1±6.1	20.2±16.7ab	57.7±15.8
No amendment (Negative control)	20.5±6.3	3.8±4.0	33.4±19.6b	57.3±24.5
Mean	**21.7**	**4.7**	**20.6**	**55.2**
Effects		***P Values***		
Soil amendments	0.112	0.078	0.010**	0.951
Seed treatment	0.639	0.060	0.701	0.008**
Season	0.001***	0.007**	0.018**	0.000***
Seed trt*Soil amend	0.571	0.145	0.009**	0.730
Soil amend*Season	0.608	0.345	0.652	0.736
Seed treatment*Season	0.001***	0.389	0.053*	0.563
Seedtrt*Soilamend*Season	0.281	0.893	0.014**	0.996

Means within the same column followed by the same letter are not significantly different according Tukey's HSD mean separation test. The ± refer to standard deviation.
*Significant at P=0.05, **significant at p=0.01; ***significant at p=0.001, trt; treatment

The reinforcement of NPK with Black Majik which gave higher yields in this study may be due to the presence of humic substances in Black Majik. Humic substances neutralize the soil pH,

acting as a pH buffer and thereby allowing availability of trace elements to the plants as reported by Pettit (2012). Humic acid has been shown to consistently increase yields when used together with a phosphatic fertilizer (P_2O_5) (Hopkins and Stark, 2003; Selim *et al*., 2010). These results concur with earlier findings indicating that the reinforcement of an inorganic fertilizer with organic amendment increases potato yields (Lemaga *et al.,* 2001; Powon *et al.,* 2005; Nakhro and Dkhar, 2010; Eufemio *et al.,* 2013).

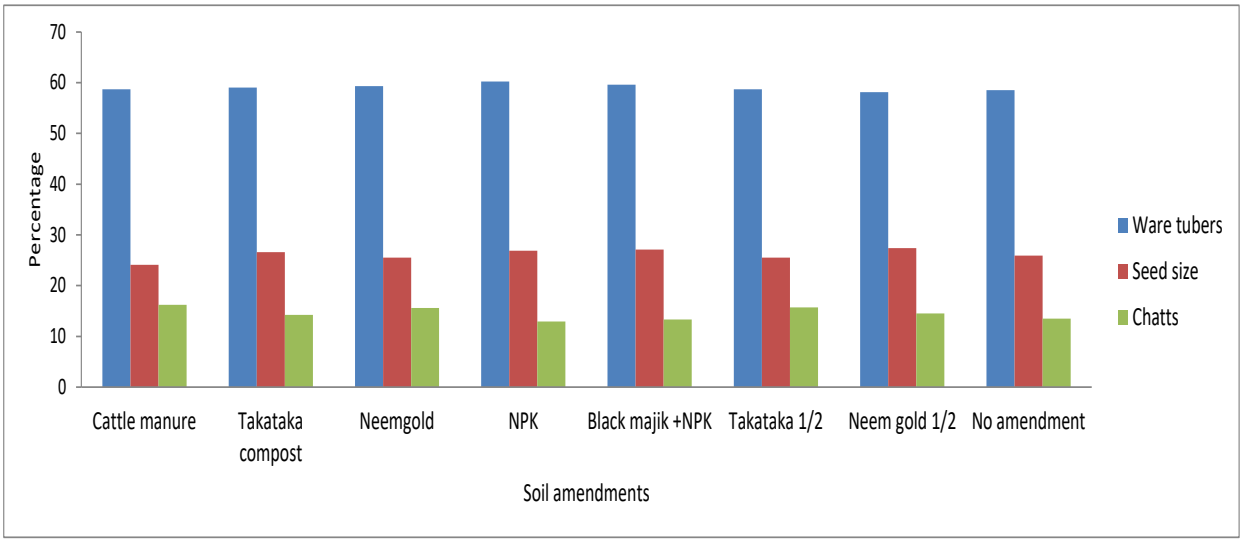

Figure 5. 3: Effect of soil organic amendments on the percentage yield of ware, seed and chatts tubers in Egerton site

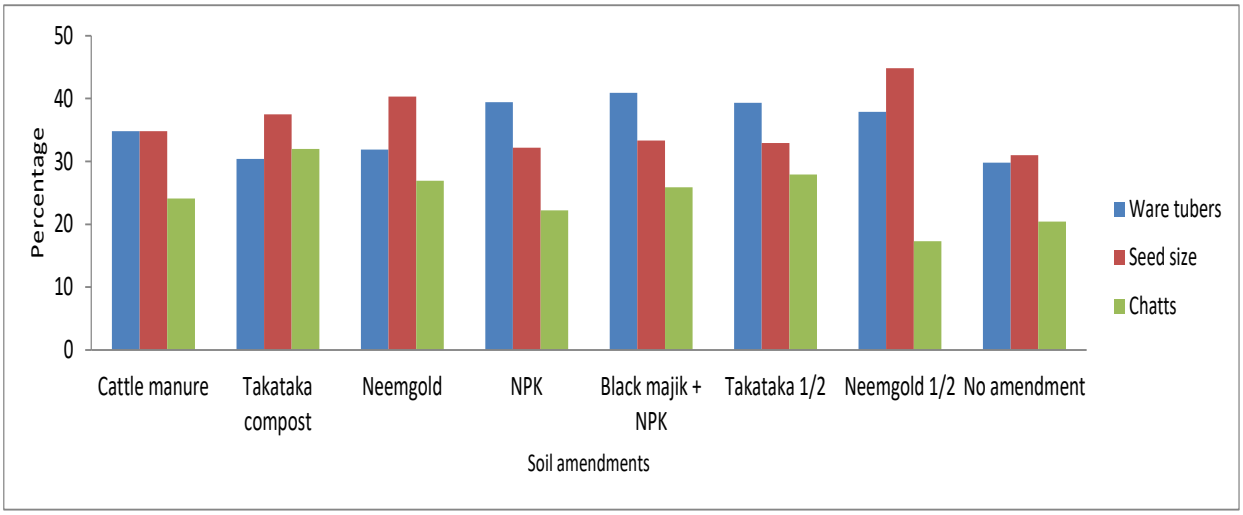

Figure 5. 4: Effect of soil organic amendments on the percentage yield of ware, seed and chatts tubers in KARLO site

5.4.3 Effect of seed treatment with Cleanstart on percent wilting incidence and yield

Seed treatment did not result in any significant effect on any of the variables analyzed and was significantly influenced by seasons. This may be attributed to the total rainfall and temperature within the duration of the study (Figure 5.1 and 5.2). The effects of Cleanstart were inconsistent between locations. As shown in Table 5.4, seed treatment did not indicate a positive significant effect on the yield and wilting incidence compared to the seeds which were not treated with Cleanstart. Cleanstart is recommended for shielding plants from *R. solanacearum* causing bacterial wilt in potatoes (www.juancogroup.com). Its ineffectiveness in this study may be attributed to factors such as the bacterial population in the soil and duration of dipping tubers in the solution among other factors.

Other studies have also shown that not all seed treatments are effective in controlling bacterial wilt. Ghosh and Mandal (2009) evaluated several antibiotics against bacterial wilt in potatoes and the results showed that organomercurials, carbendazim and streptocycline did not protect potato plants from bacterial infection The authors also observed that further use of Biovita (*Ascophyllum nodosum*); sea weed extract in seed piece tuber treatment may have predisposed the host, scavenged the effect of the antibiotics or enhanced bacterial multiplication. Kuarabachew *et al.* (2007) demonstrated that some strains of *Pseudomonas fluorescens* evaluated for *in vitro* inhibition to *R. solanacearum* had some antibiosis effect on the pathogen. Treatment with some of the *P. fluorescens* strains also suppressed disease incidence in the greenhouse and also increased the survival rates of the plants. Other BCAs (Ω5.1) and (*Pseudomonas resinovorans*) assessed against bacterial wilt in potato have indicated significant reduction in the disease levels (Smith, 2000).

Table 5.4: Response of percent wilting incidence and yield to seed treatment with cleanstart and soil amendment in pair wise comparison to the no amendment (negative control).

With CS	Soil amendment	Yield	WI	Ware ≥ 55mm	Seed size 35-55mm	Chatts ≤ 35
	Cow manure	12.7 ±10.2b	44.1±25.9	47.7±16.1a	30.6±12.3	21.6±11.7
	Takataka Compost	14.0±10.2b	37.2±23.3	40.6±13.3a	31.7±9.3	17.7±10.4
	Neemgold	11.3±9.6b	38.4±32.1	44.9±26.0a	34.0±20.6	21.1±19.7
	NPK	16.4±11.3a	39.2±25.4	50.9±15.5b	33.4±9.3	15.7±7.6
	Black majik + NPK	13.4±11.7b	36.8±35.2	49.4±18.9a	30.7±13.3	20.0±23.1
	Takataka ½	12.3±11.5b	47.5±27.1	46.2±23.2a	31.3±21.5	22.6±23.2
	Neemgold ½	11.2±9.1b	37.5±31.0	46.6±22.5a	33.5±19.5	19.9±17.7
	No amendment	12.9±12.2b	41.8±28.2	41.2±27.7a	26.1±16.0	19.7±20.0
Mean		**13.0**	**40.3**	**47.2**	**31.4**	**19.7**
Lsd values		1.7*	Ns	8.9*	Ns	Ns
Without CS	Cow manure	14.3 ±11.2a	26.4±23.4a	46.8±19.2a	28.3±12.4	18.7±8.7
	Takataka Compost	11.5±9.1a	34.2±30.1a	39.0±24.9b	32.5±14.9	28.6±22.8
	Neemgold	12.4±12.4a	34.6±27.1a	46.3±16.6a	31.3±10.1	21.5±12.0
	NPK	16.1±11.8b	38.7±25.1b	48.6±20.6a	25.7±9.5	19.5±15.6
	Black majik + NPK	14.6±9.2a	33.6±27.5a	51.2±19.1a	29.6±11.8	19.3±11.6
	Takataka ½	12.9±9.6a	32.8±21.1a	51.9±18.6a	27.1±14.0	21.0±8.6
	Neemgold ½	12.1±12.2a	34.8±23.6a	49.4±18.2a	38.7±17.4	11.9±9.5
	No amendment	11.5±7.4a	48.9±21.6b	48.521.0a	31.0±14.0	14.3±10.5
Mean		**13.2**	**35.5**	**47.7a**	**30.5**	**19.3**
Lsd values		3.8*	12.3*	8.5*	Ns	Ns

Ns: Treatments are not significant, or *- Significant at P<0.05.
Means followed by the same letter within the same column are not significantly different according to LSD test at 5%. The ± refer to Standard deviation. With CS: Seed treated with Cleanstart, Without CS: Seed not treated with Cleanstart

5.4.4 Effect of soil amendments on pH, ttotal nitrogen, pphosphorus and ttotal organic carbon on yield and bacterial wilt incidence

The study also tested the effect of the amendments used on the pH, TN, TOC and P at maturity stage to determine their residue contribution to the next crop. The results showed there were no significant differences at P=0.05, due to the soil amendments on the levels of N, P, OC and pH at the maturity stage (Appendix 10). Similar results were also observed by Laczano *et al.* (2012) between different amendments on the total organic carbon at the harvest stage of sweet corn. The

quality of soil amendments is reported to have a low influence in the late stages of crop growth and most of the decomposition at the later stages are controlled by other factors such as climate, soil texture, etc. (Mohanty *et al.,* 2013). However there were significant differences on the levels of the soil biochemical properties between the two sites (Appendix 10). The levels of TOC and TN were significantly lower ($P<0.001$) in KALRO site compared to Egerton site. The Phosphorus (ppm) was also significantly higher ($P<0.001$) and soils more acidic ($P<0.05$) in KALRO site compared to Egerton site. The results also indicated strong linear relationships between the biochemical variables analyzed, yield and wilting incidence as indicated in Table 5.5.

Multiple regression analysis was used to test if the soil biochemical variables at the maturity stage predicted the yield of the potatoes and wilting incidence. The results of the regression indicated the four predictors (TOC, TN, P, pH) explained 87.2% of the variance [R^2 =0.872, F (4, 63) = 108.3, P<.001] in yield. The pH also significantly predicted yield (β= 0.349, P< .001) as did the total organic carbon (β= 0.808, P< = .01). The results also indicated that the variables explained 68% of the wilting incidence [R^2 =0.679, F (4, 63) =34.39, P< .001]. The pH also played a significant role in predicting the wilting incidence at β = 0-.25 (P< 0.05).

The different levels of the soil bio chemical variables indicated significant relationships with wilting incidence and yield at $P<0.001$ except for phosphorus according to table 5.5. The differences observed in the yield, wilting incidence and TN, OC, pH, and P at the later stage in this study may have been due to multiple and complex biotic and abiotic interactions such as the soil type and pH, quality of the soil amendments soil moisture and climate (Mohanty *et al.,* 2013; Yuliar and Koki, 2015). A low pH which means acidic soils in KALRO site contributed to the low yields which may have been due to decreased microbial biomass. Very acidic soils have been reported to lower microbial activity and numbers (Ramesh and Bandyopadhyay, 1993; Janse, 1996; Mc Cauley *et al.,* 2009) and therefore low yields. Adequate total organic carbon and N at the growth stages implies more microbial activity, active decomposition and more mineralization of N for the crop. There was a positive correlation between TOC and TN which also correlated positively with pH towards a value of 6.51 (note the soil tested was within a range of 4.79 to 6.51). The relative increase in the TOC in relation to TN may indicate a stable C: N ratio in the soil thereby adequate mineralization which translates to availability of microbial

energy source and soluble inorganic N respectively and therefore the increase in yield. A negative correlation of TOC and wilting observed may also indicate that TOC had a significant effect on the response of the crop to disease infection and may not necessarily mean suppression of *R. solanacearum*. Laczano *et al.* (2012) observed that incorporation of organic carbon resulted to an increase in gram negative bacteria. This indicates that an increase in TOC may not have necessarily suppressed the gram negative pathogen but may have played a role in boosting the plants vigour. The soil carbon provides energy source for mineralization to take place and the subsequent inorganic N availability to microbial biomass for the synthesis of nucleic acids, proteins and other organic constituents (Zahir, 2014). This boosts the microbial community inclusive of the existing pathogens which causes competition and consequently suppression of the pathogen effect. The adequate availability of TOC and N also boosts the plant health and increases the resistance of the hosts to the pathogen. This is also observed in that TN correlated negatively with wilting incidence of the crop.

Table 5. 5: Pearson Correlation coefficients between yield, wilting incidence, pH, Nitrogen, Organic carbon and phosphorus levels in the soil at maturity stage of potatoes grown in bacterial wilt sick plots at Egerton and KALRO

	Total Nitrogen	Phosphorus (ppm)	Total Organic carbon	Yield (tons/ha)	Wilting Incidence
pH(4.79 – 6.51)	0.679**	- 0.409**	0.674**	0.803**	- 0.710**
Total Nitrogen		- 0.188	0.987**	0.884**	- 0.853**
Phosphorus (ppm)			- 0.216	- 0.343**	0.211
Total OC				0.896**	- 0.848**
Yield (tons/ha)					- 0. 831**

**. Correlation is significant at the 0.01 level (2-tailed).
OC-Organic Carbon

5.5 Conclusions and recommendations

High rainfall and low pH are important factors that encourage bacterial wilt in potato production therefore reducing yields. Strongly acidic soils (<5) are not conducive for potato production since they will lead to low yields and high losses due to bacterial wilt in soils infested with *R.*

solanacearum. This study indicates that a pH of 5.5- 6.5 is optimum for potato production as it increases availability of most micro and macro nutrients in plants. The study shows that organic fertilizers are not enough to provide the nutrients required by the potato plant in the short term and reinforcement of inorganic fertilizers with organic amendment increases potato yields. NPK + Black majik is a promising combination of organic and inorganic fertilizer which can be used to increase yields in the short term and also improve reduce bacterial wilt incidence. NPK + Black majik, neemgold and takataka compost are also promising treatments in control of bacterial wilt.

The different levels of the soil bio chemical variables indicated significant differences in the wilting incidence and yield. It is therefore important to maintain adequate nutrients during the different growth stages of potato. According to the study, the total organic carbon, total nitrogen and pH even at later stages of growth are important factors in the plants vigour and response to bacterial wilt infection. Although these soil biochemical variables analyzed showed linear relationships, it is important to note that addition of mineral fertilizers does not necessarily mean a continuous increase in yield since excess of these inorganic nutrients leads to toxicity and therefore at a certain point results to decrease in yields. Further research should be carried out to evaluate the effect of different combinations of locally available organic and inorganic inputs on bacterial wilt in potatoes. This study therefore recommends production of potato in environments with optimum requirement for production such as temperature and more important pH. The use of Cleanstart should be evaluated on soils with different levels of inoculum and the duration of seed dressing should also be considered with different potato varieties and with different soils. It is also important to determine the response of seed potato dressed with Cleanstart in different rainfall seasons.

CHAPTER SIX

GENERAL CONCLUSIONS AND RECOMENDATIONS

The purpose of this study was to establish the prevalence, incidence and distribution of potato bacterial wilt in Nakuru County. The role of crop rotation and soil amendments on bacterial wilt and yields of potato was further tested. The study showed that bacterial wilt is prevalent in Nakuru County, however the highest mean incidence recorded was 41%. Bacterial wilt in the County is distributed across the altitudes within the potato producing areas. The main factors contributing to its spread are seed sources, variety grown and use of degenerated seed. The existence of biovar 2 from bitter sweet nightshade and biovar 3 from purslane weed requires farmers to weed them out since they could be alternative hosts of the pathogen. Pre-crops of wheat-spring onion and spring onion-barley significantly reduced bacterial wilt in Egerton site and can be recommended to farmers to reduce bacterial wilt in their farms. NPK + Black majik, neemgold and takataka compost were also effective in reducing bacterial wilt incidence and increasing yields and therefore can be recomendeded to farmers. Combining organic and inorganic fertilizers is important in reducing bacterial wilt compared to sole application of organic or inorganic fertilizers. Soil pH is an impotant factor to consider in potato production especially in soils infested with the pathogen. An integrated use of the recommended crop rotation sequences and soil amendments will contribute to reduced bacterial wilt and increased yields

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APPENDICES

Appendix 1: Questionnaire for assessment of cropping practices and bacterial wilt in potato production systems in Nakuru County

Questionnaire No…… Date of interview: ……..……*day/month/year*

Enumerator's name ……

Location of survey site

County…… Ward…….……

Location……. Sub- location ……..…Village…….……..

GPS readings …… /……..…… / …..…….

Altitude (metres) *Longitude (East)* *Latitude (N or S)*

Household Characteristics

1. Respondent's name …… Farmer's name …….……

(If different from the respondent)

2. Farmer's gender (tick): Male [ ] Female [ ]

3. Farm size …… (Ha/acres) Area under potatoes…… (Acres)

4. What was the yield of potato last season ……. (Bags per acre).

5. How much was sold?.....….. (Bags) Price per bag (KShs.)……..…

Farming practices and constraints

6. Is the current crop for seed or ware? _____

7. Varieties grown _____, _____, _____

8. Age of the current potato crop in weeks _____ planting date indication _____

9. Which field generation are the current potato crops? _____, _____

10. Up to how many generations do you grow before sourcing for new seed? _____

11. Where do you get potato seed tubers for planting?

Buy from KARI [ ] Neighbour [ ] own seed [ ] Market [ ] Others ……

12. What pests do you encounter in potato production? ……

…….

13. Do you grow any other crops in your farm? .....

14. Which other crops do you grow?

.....

15. How many seasons have you grown these other crops to the current potato crop?

.....

16. Among the crops grown, which has the highest financial returns in order of priority?

.....

17. What type of potato diseases do you know? Viral [ ] Fungal [ ] Bacterial [ ] others [ ]

……..

18. Are there any diseases that can be spread through seed potato tubers? Yes [ ] No [ ] I do not know [ ]

If yes, which ones do you know of?

.....

19. Any diseases/pests that you have heard about but not in your farm.

Diseases/Pest type (1. Aware (which one), 2. Not aware)
Virus:
Blight:
Nematode:
Bacterial wilt:
Aphids:
Rodents:

20. Do you incur any losses due to bacterial wilt.....

21. Approximately, what amount per sacks harvested is infected with bacterial wilt?

.....

Bacterial wilt incidence record

For each farm site, identify 4 different parts/portions (A, B, C & D) of a potato field and record the number of bacterial of wilt affected plants.

Portion code	**Dimensions of assessed portion (metres x metres)**	**Age or stage of potato crop growth**	**No. of potato plants with BW symptoms**	**Plant population** (Total number of plants in assessed portion)	**Other comments or observations**
A					
B					
C					
D					

Appendix 2: Table showing differentiation of *Ralstonia solanacearum* biovars based on utilization of various carbon sources

Biochemical used		**Biovars**				
		1	**2**	**3**	**4**	**5**
Hexose alcohol	Mannitol	**-**	**-**	**+**	**+**	**+**
	Sorbitol	**-**	**-**	**+**	**+**	**-**
	Dulcitol	**-**	**-**	**+**	**+**	**-**
Disaccharides	Lactose	**-**	**+**	**+**	**-**	**+**
	Maltose	**-**	**+**	**+**	**-**	**+**
	Cellobiose	**-**	**+**	**+**	**-**	**+**
Positive Control	Dextrose	**+**	**+**	**+**	**+**	**+**
Negative Control	Salicin	**-**	**-**	**-**	**-**	**-**

Appendix 3: GPS coordinates and bacterial wilt incidence of fields sampled

SAMPLING SITES	ALT (M ASL)	BWI (%)	LONGITUDE	LATITUDE
MAUCHE				
Farm 1	2366	0.0	035°, 59. 572	00°, 30. 613
Farm 2	2403	0.0	035°, 00. 459	00°, 31. 898
Farm 3	2406	6.1	035°, 58. 464	00°, 29. 353
Farm 4	2411	0.0	035°, 59. 867	00°, 31. 929
Farm 5	2422	0.0	035°, 58. 811	00°, 30. 218
Farm 6	2422	0.0	035°, 58. 841	00°, 30. 286
Farm 7	2434	7.3	035°, 58. 350	00°, 29.935
Farm 8	2471	0.0	035°, 57. 335	00°, 29. 165
Farm 9	2562	0.0	035°, 58. 247	00°, 31. 683
Farm 10	2575	0.0	035°, 57. 352	00°, 30. 652
Farm 11	2577	6.6	035°, 57. 195	00°, 30. 488
Farm 12	2631	0.3	035°, 57. 464	00°, 31. 811
Farm 13	2638	2.3	035°, 5 7. 313	00°, 31. 698
Farm 14	2640	0.0	035°, 56. 863	00°, 30. 614
KERINGET				
Farm 1	2511	5.5	035°, 34. 962	00°, 27. 219
Farm 2	2533	1.2	035°, 34. 805	00°, 25. 039
Farm 3	2584	9.1	035°, 35. 835	00°, 24. 286
Farm 4	2608	0	035°, 37. 085	00°, 24. 348
Farm 5	2612	0	035°, 42. 326	00°, 31. 825
Farm 6	2613	0	035°, 59. 010	00°, 33. 533
Farm 7	2635	0.3	035°,41.986	00°, 27. 930
Farm 8	2644	0	035°,41.747	00°, 27. 706
Farm 9	2646	0	035°, 42. 096	00°, 29. 869
Farm 10	2667	0	035°, 38. 152	00°, 23. 412

Farm 11	2687	0	035°, 41. 480	00°, 27. 023
Farm 12	2736	0	035°, 39. 034	00°, 21. 489
Farm 13	2749	0.3	035°, 39. 616	00°, 20. 404
Farm 14	2764	0.3	035°, 41. 413	00°, 22. 596
Farm 15	2768	0	035°, 40. 886	00°, 22. 283
Farm 16	2770	0	035°, 40. 787	00°, 22. 250
Farm 17	2612	0.0	035°, 42. 779	00°, 31. 128
MAU NAROK				
Farm 1	2520	13.5	036°, 01. 574	00°, 34.006
Farm 2	2582	0.8	035°, 59. 005	00°, 33. 528
Farm 3	2589	6.8	035°, 59. 489	00°, 35. 194
Farm 4	2592	2.1	035°, 33. 514	00°, 59. 128
Farm 5	2634	41	035°, 59. 835	00°, 35. 496
Farm 6	2684	7	036°, 00. 494	00°, 36. 447
Farm 7	2701	24.8	036°, 00. 348	00°, 36. 508
Farm 8	2736	0	036°, 00. 794	00°, 37.622
Farm 9	2875	6.2	035°, 59. 154	00°, 39. 291
Farm 10	2880	0	035°, 59. 099	00°, 40. 185
Farm 11	2882	10.4	035°, 59. 945	00°, 38. 624
Farm 12	2887	1.7	035°, 59. 543	00°, 38. 070
Farm 13	2889	0.3	035°, 59. 368	00°, 39. 867
Farm 14	2896	8.1	036°,00.006	00°, 37.787
Farm 15	2935	0.2	036°, 00. 275	00°, 38. 763
Farm 16	2936	0	036°, 00. 577	00°, 39.066
Farm 17	2942	6.7	036°, 00. 701	00°, 39. 407
KURESOI				
Farm 1	2567	5.2	035°, 00. 489	00o, 19. 185
Farm 2	2570	0.4	035°, 30. 666	00o, 19. 115
Farm 3	2608	1.2	035°, 35. 875	00o, 20. 723

Farm 4	2625	0.6	035°, 33. 858	00o, 18. 315
Farm 5	2630	2.2	035°, 32. 864	00°, 17. 754
Farm 6	2633	12.5	035°, 32. 322	00°, 17. 659
Farm 7	2656	3.1	035°, 33. 752	00°, 17. 206
Farm 8	2660	2.3	035°, 34. 882	00°, 18. 503
Farm 9	2660	8.2	035°, 36. 054	00°, 19. 251
Farm 10	2682	14.8	035°, 35. 002	00°, 18. 658
Farm 11	2683	0.4	035°, 37. 736	00°, 20. 286
Farm 12	2684	5.6	035°, 35. 266	00°, 18. 676
Farm 13	2690	0	035°, 36. 444	00°, 18. 543
Farm 14	2736	10.5	035°, 38. 087	00°, 19. 397
Farm 15	2743	0	035°, 35. 709	00°, 17. 063
ELBURGON				
Farm 1	2353	2.4	035°, 49. 963	00°, 17. 519
Farm 2	2400	0.9	035°, 49. 882	00°, 17. 126
Farm 3	2534	0	035°, 46. 647	00°, 18. 241
Farm 4	2542	0	035°, 46. 641	00°, 18. 274
Farm 5	2579	1.9	035°, 46. 439	00°, 18. 575
Farm 6	2600	0.8	035°, 46. 651	00°, 18. 335
Farm 7	2633	0.9	035°, 46. 739	00°, 19. 524
Farm 8	2639	6.9	035°, 46. 809	00°, 19. 500
Farm 9	2655	0	035°, 48. 758	00°, 20. 943
Farm 10	2656	0	035°, 44. 796	00°, 18. 984
Farm 11	2680	8.3	035°, 47. 207	00°, 20. 793
Farm 12	2700	1.2	035°, 47. 284	00°, 21. 293
Farm 13	2717	0	035°,39.084	00°, 33.726
Farm 14	2717	0.2	035°, 46. 203	00°, 21. 419
Farm 15	2718	0.2	035°, 46. 518	00°, 20.655
Farm 16	2729	8.3	035°, 49. 001	00°, 22. 763
Farm 17	2743	7.1	035°, 47. 916	00°, 22. 838

Farm 18	2793	0	035°, 47. 325	00°, 22. 251
KAMARA				
Farm 1	2483	0.7	035°, 42. 972	00°, 11. 472
Farm 2	2492	0.6	035°, 40.585	00°, 09. 833
Farm 3	2500	0	035°, 43. 378	00°, 10. 558
Farm 4	2508	0	035°, 40. 278	00°, 10. 561
Farm 5	2520	5.5	035°, 43. 293	00°, 10. 275
Farm 6	2532	6.8	035°, 40. 576	00°, 10. 236
Farm 7	2562	0.3	035°, 43. 483	00°, 09. 672
Farm 8	2576	2.2	035°, 40.902	00°, 07. 054
Farm 9	2578	1.4	035°, 41. 556	00°, 12. 321
Farm 10	2581	0	035°, 40. 737	00°, 09. 130
Farm 11	2581	3.9	035°, 40. 419	00°, 09. 031
Farm 12	2598	1.6	035°, 41. 451	00°, 12. 303
Farm 13	2602	0.3	035°, 41. 389	00°, 07. 906
Farm 14	2623	0	035°, 42. 594	00°, 09. 409
Farm 15	2634	0.3	035°, 41. 134	00°, 07. 434
Farm 16	2688	0	035°, 43. 087	00°, 09. 049
Farm 17	2733	1.5	035°, 42. 965	00°, 08. 435
Farm 18	2759	18	035°, 42. 265	00°, 06. 744
MOLO				
Farm 1	2455	0	035°, 43. 697	00°, 12. 682
Farm 2	2467	0.3	035°, 45. 604	00°, 15. 552
Farm 3	2492	1.9	035°, 45. 803	00°, 15. 887
Farm 4	2536	2.5	035°, 45. 905	00°, 17. 936
Farm 5	2583	3.3	035°, 42. 115	00°, 13. 870
Farm 6	2588	6.9	035°, 42. 783	00°, 15. 289
Farm 7	2641	0	035°, 43. 422	00°, 17. 220
Farm 8	2680	0	035°, 42. 683	00°, 16. 972
Farm 9	2696	0	035°, 42. 004	00°, 18 .350

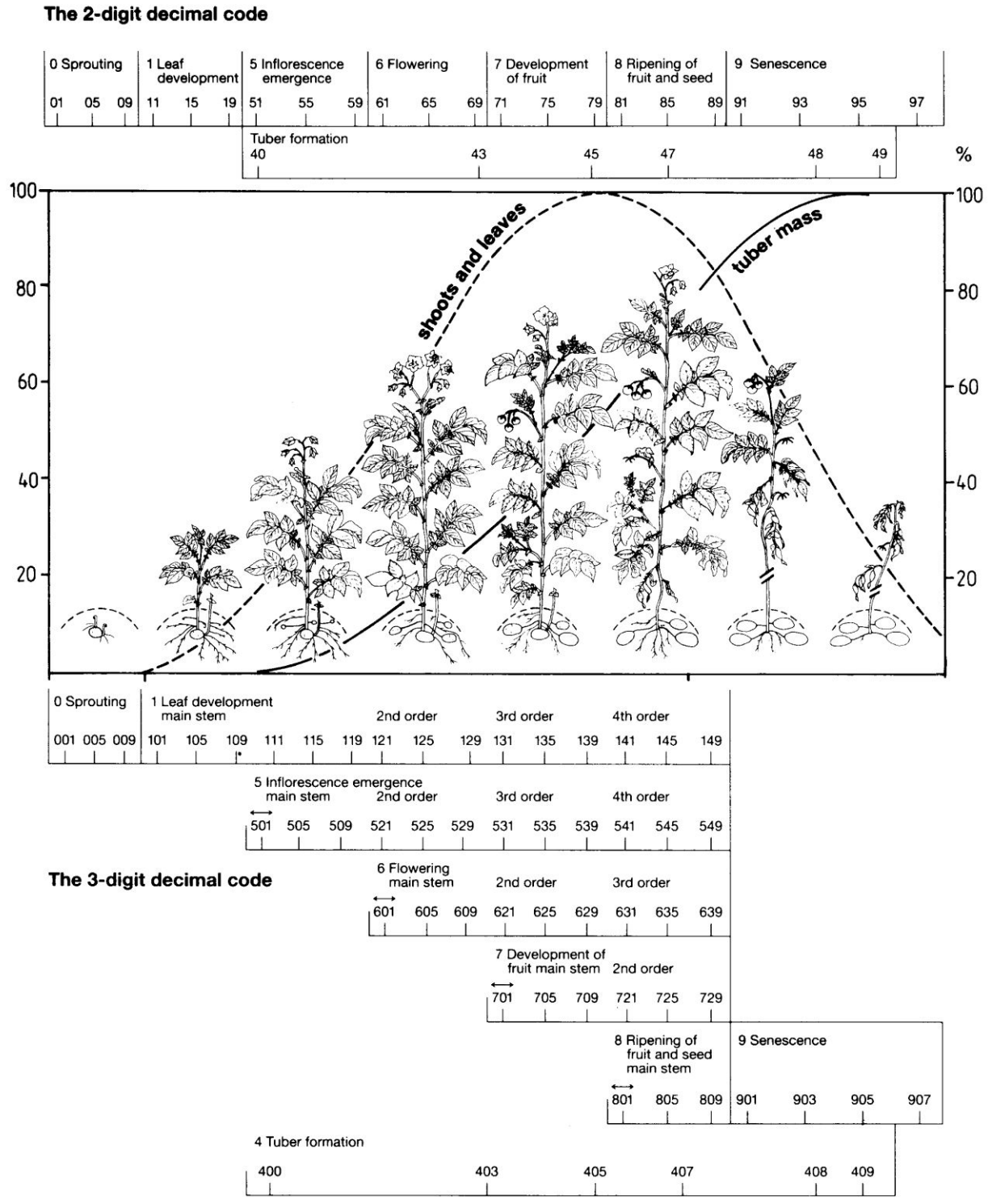
Farm 10	2703	0.2	035°, 42. 554	00°, 17. 112
Farm 11	2708	0	035°, 42. 206	00°, 17. 701
Farm 12	2716	0	035°, 42. 820	00°, 17. 601
Farm 13	2727	0.4	035°, 41. 190	00°, 18 974
Farm 14	2727	8.3	035°, 41. 190	00°, 18 974
Farm 15	2745	0	035°, 41. 795	00°, 18 995
Farm 16	2781	0	035°, 41. 487	00°, 19. 334
OLENGURUONE				
Farm 1	2495	0	035°,39.072	00°, 33.714
Farm 2	2506	5.8	035°, 40. 432	00°, 36. 482
Farm 3	2517	2.3	035°,40.788	00°, 35. 092
Farm 4	2547	0.7	035°, 42. 343	00°, 34.255
Farm 5	2548	12.5	035°, 39. 942	00°, 32. 940
Farm 6	2553	15.8	035°,41.952	00°, 35. 078
Farm 7	2563	10	035°, 41. 894	00°, 35. 052
Farm 8	2582	4.7	035°, 45. 155	00°, 32. 836
Farm 9	2583	18.6	035°, 41. 696	00°, 32. 310
Farm 10	2584	0.3	035°, 41. 685	00°, 34. 801
Farm 11	2589	24	035°, 43. 177	00°, 34. 362
Farm 12	2589	42	035°, 41. 097	00°, 32. 815
Farm 13	2590	6.6	035°, 42. 877	00°, 34. 974
Farm 14	2591	0.3	035°, 41. 650	00°, 34. 433
Farm 15	2593	4.9	035°, 41. 472	00°, 34. 079
BAHATI				
Farm 1	2032	13.6	036°, 08. 889	00°, 11.876
Farm 2	2037	9.2	036°,10.165	00°, 13.173
Farm 3	2050	0.8	036°, 09. 922	00°, 12.844
Farm 4	2054	7.2	036°, 09. 906	00°, 09. 906
Farm 5	2142	2.7	036°, 09. 937	00°, 09. 409
Farm 6	2145	13.7	036°, 10.010	00°, 09.361

Farm 7	2145	4.9	036°,10.377	00°, 11.170
Farm 8	2159	7.7	036°,09.874	00°, 08.415
Farm 9	2161	4.7	036°,09.912	00°, 08.425
Farm 10	2166	0.9	036°,09.829	00°, 08. 220
Farm 11	2168	6.25	036°, 12.357	00°, 15. 156
Farm 12	2233	0.8	036°, 11. 074	00°, 09.611
Farm 13	2245	0.3	036°, 11. 254	00°, 09.274
Farm 14	2252	4.9	036°, 11. 259	00°, 09.366
Farm 15	2344	3.8	036°, 11. 695	00°, 08.532

Appendix 4: Basal medium preparation for *R. solanacearum* biovar testing

Ingredients	**1000 ml**	**700 ml**
Ammonium dihydrogen phosphate ($NH_4H_2PO_4$	1.0 g	0.7 g
Potassium Chloride (KCL)	0.2 g	0.14 g
Magnesium sulphate ($MgSO_4.7H_2O$	0.2 g	0.14 g
Peptone	1.0 g	0.70 g
Bromothymol blue	0.03 g	0.021 g
Agar	3.0 g	2.10 g
Water	1.0 g	700 ml

Appendix 5: Phenological growth stages and BBCH-identification keys of potato



Appendix 6: CPG medium for cultivation of *Ralstonia solanacearum*

Casamino acids (Difco	1.0g
Bacto-Peptone (Difco)	10.0 g
Glucose	10.0 g
Bacto-Agar (Difco)	18.0 g
Distilled water	100 ml

Autoclave the above ingredients at 121ºC for 15 minutes and cool to about 40-45ºC before pouring.Pour the homogenized medium into sterile Petri dishes.

Source; Kinyua et al., (2014)

Appendix 7: Ingredients for preparation of semi-selective medium, SMSA

	For 1000ml	***For 500ml***	***For 250ml***
Casamino acids (Difco)	1.0g	0.5g	0.25g
Bacto-Peptone (Difco)	10.0g	5.0g	2.50g
Glycerol	5.0ml	2.5ml	1.25ml
Bacto-Agar (Difco)	15.0g	7.5g	3.75g
Distilled water	1000ml	500ml	250ml

Additive	**Stock solution concentration**	**Required concentration in basal medium**	**Amount to add to basal medium**
			500 ml
Crystal violet (sigma)	1% (0.1g in 10mls water)	5mg/litre	250µl
Chloramphenicol (Sigma C-3175)	1% (0.1g in 10mls Methanol)	5mg/litre	250µl
Penicillin G (Sigma P-3032)	0.1% (0.01g in 10mls water)	0.5mg/litre	250µl
Bacitracin (Sigma B-0125)	1% (0.1g in 10mls Methanol)	25mg/litre	1250µl
Tetrazolium salts (Sigma)	1% (0.1g in 10mls water)	50mg/litre	2500µl
Polymixin B Sulphate (Sigma P-1004)	1% (0.1g in 10mls water)	100mg/litre	5000µl

Source; Kinyua et al., (2014)

Appendix 8: Bacterial density in the crop rotation experiment

						(cfu x 10^4/gram)
SITE	**REP**	**ROTATION TYPE**	**CROP SEQUENCES**	**BP IS**	**BPS1**	**BPS2**
KALRO	1	One crop rotation	Potato Lablab Potato	200	15	0
KALRO	1	One crop rotation	Potato Potato Potato	180	0	0
KALRO	1	One crop rotation	Potato Canola Potato	184	3	5
KALRO	1	One crop rotation	Potato GardenPea Potato	196	7	4
KALRO	1	One crop rotation	Potato Barley Potato	280	3	0
KALRO	1	One crop rotation	Potato Cabbage Potato	13	2	14
KALRO	2	One crop rotation	Potato Cabbage Potato	292	5	22
KALRO	2	One crop rotation	Potato Wheat Potato	33	7	3
KALRO	2	One crop rotation	Potato Potato Potato	288	3	18
KALRO	2	One crop rotation	Potato Spring Onion Potato	264	3	18
KALRO	2	One crop rotation	Potato Barley Potato	297	15	4
KALRO	2	One crop rotation	Potato Canola Potato	228	3	14
KALRO	2	One crop rotation	Potato GardenPea Potato	84	3	7
KALRO	3	One crop rotation	Potato Barley Potato	16	23	19
KALRO	3	One crop rotation	Potato Wheat Potato	60	11	9
KALRO	3	One crop rotation	Potato Lablab Potato	128	5	21
KALRO	3	One crop rotation	Potato GardenPea Potato	128	27	0
KALRO	3	One crop rotation	Potato Potato Potato	293	7	21
KALRO	3	One crop rotation	Potato Canola Potato	305	3	6
KALRO	3	One crop rotation	Potato Cabbage Potato	65	5	7
KALRO	4	One crop rotation	Potato Cabbage Potato	298	2	22
KALRO	4	One crop rotation	Potato Canola Potato	49	6	23
KALRO	4	One crop rotation	Potato Spring Onion Potato	25	6	21
KALRO	4	One crop rotation	Potato Potato Potato	9	0	7
KALRO	4	One crop rotation	Potato Barley Potato	7	0	8
KALRO	4	One crop rotation	Potato Wheat Potato	144	2	9
KALRO	3	One crop rotation	Potato Spring Onion Potato	24	3	11

KALRO	4	One crop rotation	Potato GardenPea Potato	14	9	7
KALRO	1	One crop rotation	Potato Spring Onion Potato	26	0	11
KALRO	1	One crop rotation	Potato Wheat Potato	208	11	10
KALRO	2	One crop rotation	Potato Lablab Potato	248	11	9
KALRO	4	One crop rotation	Potato Lablab Potato	23	21	0
KALRO	1	Two precrops rotation	Spring Onion Barley Potato	223	9	7
KALRO	1	Two precrops rotation	Garden Pea Canola Potato	284	5	0
KALRO	1	Two precrops rotation	Fallow Fallow Potato	144	3	7
KALRO	1	Two precrops rotation	Spring Onion Wheat Potato	284	1	8
KALRO	1	Two precrops rotation	Wheat Springonion Potato	252	3	6
KALRO	1	Two precrops rotation	Barley Springonion Potato	316	9	5
KALRO	1	Two precrops rotation	Canola GardenPea Potato	74	3	3
KALRO	1	Two precrops rotation	Lablab Cabbage Potato	357	23	7
KALRO	1	Two precrops rotation	Cabbage Lablab Potato	80	7	0
KALRO	2	Two precrops rotation	Fallow Fallow Potato	378	6	7
KALRO	2	Two precrops rotation	Spring Onion Barley Potato	354	27	6
KALRO	2	Two precrops rotation	Lablab Cabbage Potato	228	9	5
KALRO	2	Two precrops rotation	Canola GardenPea Potato	144	5	17
KALRO	2	Two precrops rotation	Wheat Spring onion Potato	288	9	6
KALRO	2	Two precrops rotation	Garden Pea Canola Potato	80	2	16
KALRO	2	Two precrops rotation	Barley Springonion Potato	398	3	
KALRO	2	Two precrops rotation	Spring Onion Wheat Potato	68	3	9
KALRO	3	Two precrops rotation	Spring Onion Wheat Potato	284	4	6
KALRO	3	Two precrops rotation	Canola GardenPea Potato	28	0	0
KALRO	3	Two precrops rotation	Spring Onion Barley Potato	12	0	0
KALRO	3	Two precrops rotation	Fallow Fallow Potato	6	3	0
KALRO	3	Two precrops rotation	Lablab Cabbage Potato	13	0	0
KALRO	3	Two precrops rotation	Barley Springonion Potato	11	5	14
KALRO	3	Two precrops rotation	Wheat Springonion Potato	6	7	8
KALRO	3	Two precrops rotation	Cabbage Lablab Potato	4	5	8
KALRO	3	Two precrops rotation	Garden Pea Canola Potato	4	3	0

KALRO	4	Two precrops rotation	Cabbage Lablab Potato	2	8	13
KALRO	4	Two precrops rotation	Garden Pea Canola Potato	2	5	41
KALRO	4	Two precrops rotation	Spring Onion Wheat Potato	9	3	0
KALRO	4	Two precrops rotation	Fallow Fallow Potato	13	3	0
KALRO	4	Two precrops rotation	Barley Springonion Potato	7	3	14
KALRO	4	Two precrops rotation	Lablab Cabbage Potato	8	3	15
KALRO	4	Two precrops rotation	Spring Onion Barley Potato	45	9	0
KALRO	4	Two precrops rotation	Wheat Springonion Potato	36	3	7
KALRO	4	Two precrops rotation	Canola GardenPea Potato	32	3	13
KALRO	2	Two precrops rotation	Cabbage Lablab Potato	44	21	18
EGERTON	1	One crop rotation	Potato Lablab Potato	10	0	51
EGERTON	1	One crop rotation	Potato Potato Potato	12	0	46
EGERTON	1	One crop rotation	Potato Canola Potato	19	0	53
EGERTON	1	One crop rotation	Potato GardenPea Potato	14	0	41
EGERTON	1	One crop rotation	Potato Barley Potato	30	0	39
EGERTON	1	One crop rotation	Potato Cabbage Potato	16	3	42
EGERTON	2	One crop rotation	Potato Cabbage Potato	16	2	47
EGERTON	2	One crop rotation	Potato Wheat Potato	26	3	43
EGERTON	2	One crop rotation	Potato Potato Potato	27	2	51
EGERTON	2	One crop rotation	Potato Spring Onion Potato	29	7	54
EGERTON	2	One crop rotation	Potato Barley Potato	23	4	68
EGERTON	2	One crop rotation	Potato Canola Potato	41	5	79
EGERTON	2	One crop rotation	Potato GardenPea Potato	3	1	83
EGERTON	3	One crop rotation	Potato Barley Potato	19	1	83
EGERTON	3	One crop rotation	Potato Wheat Potato	6	4	81
EGERTON	3	One crop rotation	Potato Lablab Potato	52	2	82
EGERTON	3	One crop rotation	Potato GardenPea Potato	18	3	87
EGERTON	3	One crop rotation	Potato Potato Potato	19	3	96
EGERTON	3	One crop rotation	Potato Canola Potato	24	10	136
EGERTON	3	One crop rotation	Potato Cabbage Potato	6	3	78
EGERTON	4	One crop rotation	Potato Cabbage Potato	39	3	104

EGERTON	4	One crop rotation	Potato Canola Potato	41	0	114
EGERTON	4	One crop rotation	Potato Spring Onion Potato	17	0	123
EGERTON	4	One crop rotation	Potato Potato Potato	18	0	127
EGERTON	4	One crop rotation	Potato Barley Potato	16	1	113
EGERTON	4	One crop rotation	Potato Wheat Potato	19	7	118
EGERTON	1	One crop rotation	Potato Spring Onion Potato	21	3	81
EGERTON	1	One crop rotation	Potato Wheat Potato	10	7	80
EGERTON	2	One crop rotation	Potato Lablab Potato	13	2	131
EGERTON	3	One crop rotation	Potato Spring Onion Potato	9	3	129
EGERTON	4	One crop rotation	Potato GardenPea Potato	0	6	71
EGERTON	4	One crop rotation	Potato Lablab Potato	7	2	130
EGERTON	1	Two precrops rotation	Spring Onion Barley Potato	3	26	72
EGERTON	1	Two precrops rotation	Garden Pea Canola Potato	23	4	95
EGERTON	1	Two precrops rotation	Fallow Fallow Potato	8	5	17
EGERTON	1	Two precrops rotation	Spring Onion Wheat Potato	0	3	13
EGERTON	1	Two precrops rotation	Wheat Springonion Potato	0	3	13
EGERTON	1	Two precrops rotation	Barley Springonion Potato	5	1	84
EGERTON	1	Two precrops rotation	Canola GardenPea Potato	5	0	106
EGERTON	1	Two precrops rotation	Lablab Cabbage Potato	20	0	101
EGERTON	1	Two precrops rotation	Cabbage Lablab Potato	0	5	106
EGERTON	2	Two precrops rotation	Fallow Fallow Potato	22	3	110
EGERTON	2	Two precrops rotation	Spring Onion Barley Potato	11	1	107
EGERTON	2	Two precrops rotation	Lablab Cabbage Potato	6	13	93
EGERTON	2	Two precrops rotation	Canola GardenPea Potato	13	7	91
EGERTON	2	Two precrops rotation	Wheat Springonion Potato	0	5	83
EGERTON	2	Two precrops rotation	Garden Pea Canola Potato	9	0	96
EGERTON	2	Two precrops rotation	Barley Springonion Potato	0	7	112
EGERTON	2	Two precrops rotation	Spring Onion Wheat Potato	12	5	114
EGERTON	3	Two precrops rotation	Spring Onion Wheat Potato	40	3	72
EGERTON	3	Two precrops rotation	Canola GardenPea Potato	25	0	106
EGERTON	3	Two precrops rotation	Spring Onion Barley Potato	15	0	87

EGERTON	3	Two precrops rotation	Fallow Fallow Potato	32	6	129
EGERTON	3	Two precrops rotation	Lablab Cabbage Potato	14	3	117
EGERTON	3	Two precrops rotation	Barley Springonion Potato	16	5	120
EGERTON	3	Two precrops rotation	Wheat Springonion Potato	0	5	124
EGERTON	3	Two precrops rotation	Cabbage Lablab Potato	18	2	67
EGERTON	3	Two precrops rotation	Garden Pea Canola Potato	17	2	93
EGERTON	4	Two precrops rotation	Cabbage Lablab Potato	0	0	97
EGERTON	4	Two precrops rotation	Garden Pea Canola Potato	0	0	28
EGERTON	4	Two precrops rotation	Spring Onion Wheat Potato	39	0	66
EGERTON	4	Two precrops rotation	Fallow Fallow Potato	15	1	81
EGERTON	4	Two precrops rotation	Barley Springonion Potato	33	6	64
EGERTON	4	Two precrops rotation	Lablab Cabbage Potato	6	3	73
EGERTON	4	Two precrops rotation	Spring Onion Barley Potato	3	3	41
EGERTON	4	Two precrops rotation	Wheat Springonion Potato	5	3	30
EGERTON	4	Two precrops rotation	Canola GardenPea Potato	17	2	72
EGERTON	2	Two precrops rotation	Cabbage Lablab Potato	9	5	49

BPS1: Bacterial Population Season 1, BPS2: Bacterial Population Season 2, CFU: Colony forming units, BPIS: Bacterial Population initial stage

Appendix 9: Bacterial wilt incidence of symptomatic plants in the survey

Growth stage	No BWI	1-10%	11-20%	21-30%	41-50%	
2	4	1	0	0	0	7
3	16	0	0	0	0	16
4	15	0	0	0	0	15
5	8	10	0	0	1	19
6	24	23	4	1	1	53
7	4	13	2	1	0	20
8	1	8	2	0	0	11
9	0	1	1	0	0	2
Total	**72**	**56**	**9**	**2**	**2**	**141**

Appendix 10: Analysis of Variance of soil macro nutrients in soil amendment experiment

Dependent Variable: pH

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	10.515[a]	31	.339	2.915	.002
Intercept	1983.589	1	1983.589	17043.887	.000
Site	8.955	1	8.955	76.946	.000
Soilamend	1.136	15	.076	.651	.811
Site * Soilamend	.425	15	.028	.243	.997
Error	3.724	32	.116		
Total	1997.829	64			
Corrected Total	14.240	63			

a. R Squared = .738 (Adjusted R Squared = .485)

Dependent Variable: Nitrogen

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.403[a]	31	.013	19.259	.000
Intercept	3.089	1	3.089	4576.009	.000
Site	.394	1	.394	583.343	.000
Soilamend	.003	15	.000	.306	.991
Site * Soilamend	.006	15	.000	.607	.847
Error	.022	32	.001		
Total	3.513	64			
Corrected Total	.425	63			

a. R Squared = .949 (Adjusted R Squared = .900)

Dependent Variable: Phosphorus

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1311.750[a]	31	42.315	.800	.731
Intercept	78680.250	1	78680.250	1488.043	.000
Site	232.563	1	232.563	4.398	.044
Soilamend	787.250	15	52.483	.993	.485
Site * Soilamend	291.938	15	19.463	.368	.978
Error	1692.000	32	52.875		
Total	81684.000	64			
Corrected Total	3003.750	63			

a. R Squared = .437 (Adjusted R Squared = -.109)

Dependent Variable: 0C

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	36.599[a]	31	1.181	23.235	.000
Intercept	309.980	1	309.980	6100.656	.000
Site	35.566	1	35.566	699.974	.000
Soilamend	.711	15	.047	.934	.540
Site * Soilamend	.321	15	.021	.421	.961
Error	1.626	32	.051		
Total	348.205	64			
Corrected Total	38.224	63			

a. R Squared = .957 (Adjusted R Squared = .916)

Appendix 11: Soil amendments nutrient composition

	Takataka compost	**Cow manure**	**Neemgold**	**NPK**	**Black majik**
Nitrogen %	2.10	1.05	1.40	17.0	1.40
Phosphorus %	0.44	0.42	0.35	17.0	0.23
Potassium %	4.70	1.81	1.83	17.0	7.40

Appendix 12: Rate of application of soil amendments per 3m x 3m plot

1. Cattle manure (7.7 kgs) + cleanstart
2. Takataka ½ (1.95 kgs)+ cleanstart
3. No amendment
4. Neem gold ½ (1.3 kgs) + cleanstart
5. Takataka compost ½ (1.95 kgs)
6. Takataka compost (3.9 kgs) + cleanstart
7. Cattle manure (7.7 kgs)
8. Neemgold (2.7 kgs)
9. NPK (476 gms) + cleanstart
10. Neemgold (2.7 kgs) + cleanstart
11. No amendment + cleanstart
12. Black majik +NPK (476 gms)
13. Black majik +NPK (476 gms)+ cleanstart
14. Takataka compost (3.9 kgs)
15. NPK (476 gms)
16. Neem gold ½ (1.3 kgs)

Appendix 13: List of Publications

Mwaniki, P. K., Birech, R., Wagara, I. N, Kinyua, Z. M, E.Schulte-Geldermann, E., .and Freyer (2016) Distribution, prevalence and Incidence of potato bacterial wilt in Nakuru County, Kenya. *International Journal of Innovative Research and Development,* 5:435-442

Mwaniki, P. K., Wagara, I. N, Birech, R., Kinyua, Z. M, E.Schulte-Geldermann, E., .and Freyer,(2017) Impact of crop rotation sequences on potato in fields inoculated with bacterial wilt caused by *Ralstonia solanacearum*. *African Journal of Agriculture,* 12: 1226-1235