EFFECT OF CANOLA (*Brassica napus* L.) GREEN MANURE AND COMPOUND FERTILIZER ON POTATO (*Solanum tuberosum* L.) GROWTH, YIELD AND BACTERIAL WILT MANAGEMENT

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A Thesis Submitted to the Graduate School in Partial Fulfillment of the Requirements for the Master of Science Degree in Agronomy of Egerton University

EGERTON UNIVERSITY

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DECLARATION AND RECOMMENDATION

Declaration

This thesis is my original work and has not been submitted or presented for examination in any other institution for the award of any degree.

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Recommendation

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DEDICATION

This thesis is dedicated to my parents and Assinapol Ndereyimana for the moral support, encouragement and guidance.

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ABSTRACT

Potato yield in Kenya is declining year by year while the demand is increasing. Bacterial wilt and poor soil fertility management are some of the major factors that cause reduction in potato yields. The objective of this study was to contribute to increased potato production through use of canola green manure in bacterial wilt management and use of compound fertilizer for soil fertility management. Three experiments were conducted in the laboratory, semi-field (pot) and field. Laboratory experiment was conducted at Egerton university biological laboratory to evaluate the effect of canola extract on *Ralstonia solanacearum* population density. Four levels of canola extract quantities were used; 0, 100, 200, 300 microlitres, and enrich immunomodulator (Di-bromo Di-nitro propane 1-3-diol) (DDD) was used as a positive control. Inoculum was isolated from infested soil on Selective Medium South Africa (SMSA) then Casamino acids, Bacto-Peptone, Glycerol, and Bacto-Agar (CPG) used as growth medium arranged in a completely randomized design. Field experiment was carried out in two sites (Elburgon site is in Upper high land zone two (UH2) and Mau-Narok is in Upper high land zone one (UH1)). Two levels of canola green manure (with and without green manure application), four levels of fertilizer (NPK+Ca+Mg+) applied at 0 (F1), 250 (F2), 575 (F3), 900 (F4) kg ha^{-1} and DAP 500 (F5) kg ha^{-1} as a positive control was used. The experiment was carried out in split-split plot design where canola green manure was main plot and fertilizer and varieties combination as sub plots. Semi-field experiment was carried out at Egerton university field farm; four levels of canola green manure (100, 75, 50 and 0 g kg^{-1} soil) and five levels of the compound fertilizer as used in the field experiment in a completely randomized design (CRD). The results showed that canola green manure had no effect on bacterial population density nor bacterial wilt incidence whereas, the interaction of fertilizer with canola green manure slightly enhanced bacterial population 2% compared to without green manure under field experiment. Treatment F4 increased potato yield and plant height by 5.0% in both cases over the recommended rate of F5. Furthermore, F4 increased nutrient (Nitrogen, phosphorus and potassium) uptake by 13%, 26% and 3% respectively under field experiment than F5. Generally, canola green manure did not show significant effect on potato plant height and yield, though F4 with green manure exhibited an increase of 7% and 38% on plant height and tuber dry weight respectively. According to the findings, farmers in Nakuru County can use (NPK+Ca+Mg+) fertilizer as alternative in potato production.

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ACRONYMS AND ABBREVIATIONS

AGRA:	Alliance for a Green Revolution in Africa
ANOVA:	Analysis of Variance
AS:	Ammonium Sulfate
ASN:	Ammonium Sulphate Nitrate
BMP:	Best Management Practice
CABI:	Centre for Agriculture and Bioscience International
CAN:	Calcium Ammonium Nitrate
CEC:	Cation Exchange capacity
Cm:	Centimeter
DAE:	Days After Emergence
DAP:	Diammonium Phosphate
EPPO:	European and Mediterranean Plant Protection Organization
FAO:	Food and Agriculture Organization of United Nations
FAOSTAT:	Food and Agriculture Organization (Statistics Division)
Ha:	Hectare
IFDC:	International Fertilizer Development Center
IPAD:	International Plants Diagnostic Network
KARI/KALRO:	Kenya Agricultural Research Institute/ Kenya Agricultural and Livestock Research Organization.
m.a.s.l:	Meter above sea level
MAP:	Mono ammonium phosphate
MoALF	Ministry of Agriculture, Livestock and Fisheries
MSD:	Minimum Significant Difference
NARL	National Agricultural Research Laboratory
NPCK:	National potato council of Kenya
NUE:	Nutrient Use Efficiency
pH:	Potential Hydrogen concentration
PSI:	Percentage severity index
RCBD:	Randomized Complete Block Design
SMSA	Selective Medium South Africa
SSA:	Sub-Saharan Africa

CHAPTER ONE

INTRODUCTION

1.1 Background information

Potato (*Solanum tuberosum* L.) is an important food crop and is the fourth most important crop after maize (*Zea mays* L.), rice (*Oriza sativa* L.) and wheat (*Triticum aestivum* L.) in global economy. It is number one non-grain food commodity (Adrien , 2013; Chemeda *et al.*, 2014). In East Africa, potato was introduced around 1880s by British farmers, as a source of food and income to the farmers (FAO, 2008a) . It contains about 79% water, 18% starch as a good source of energy, 2% protein and 1% vitamins including vitamin C, minerals such as calcium and magnesium and many trace elements (Zewide *et al.*, 2016). In East Africa the major potato producing countries are Kenya, Tanzania, Malawi, and Rwanda with high annual total production (FAOSTAT, 2020).

In Kenya, potato is the second most important food crop after maize (*Zea mays*) and is produced in the cool highlands mostly by small scale farmers, many of them who are women, although there are some larger-scale growers that specialize in commercial production (Janssens *et al.*, 2013; Potatopro, 2019). Despite its importance, there is a decline in potato production, for example, in 2009, Kenya was the highest potato producer compared to other east Africa countries but production declined up to the level it became the second last (FOASTAT, 2020) (Fig 1.1). Currently average national production is far below the potential ranging between 7,700 to 9,500 kg ha^{-1} against a potential of over 40,000 kg ha^{-1} (Komen *et al.*, 2017).

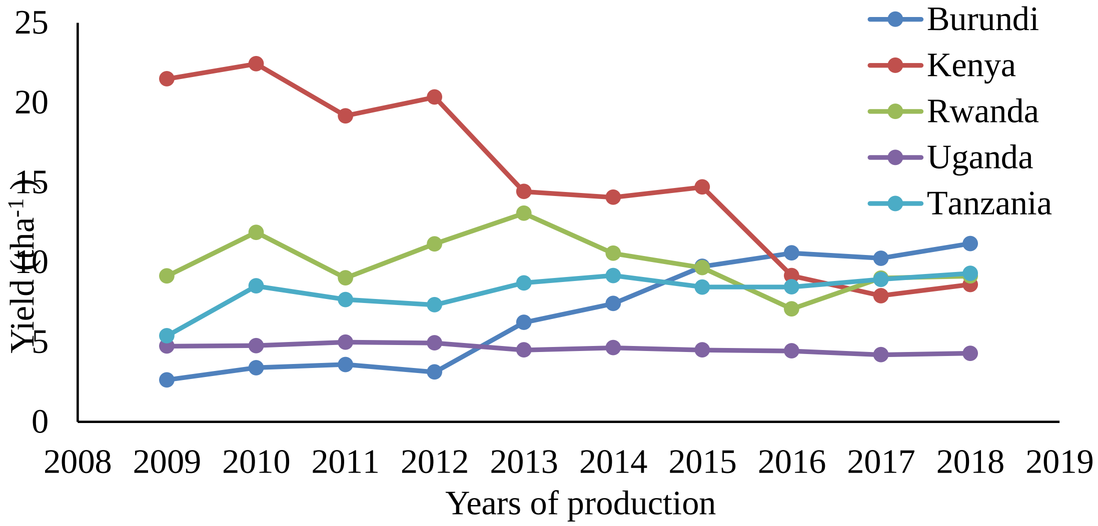


Figure 1.1: Potato production trend from 2009 to 2019 in selected countries in East Africa (FOASTAT, 2020)

Potato production in Kenya is facing many constraints; the main ones are low soil fertility and inappropriate use of fertilizer, insufficient high quality seed, poor agronomic practices (Komen *et al*., 2017). Additionally, application of fertilizers is below recommended rate of 90 kg ha^{-1} N and 230 kg ha^{-1} P_2O_5 (Kaguongo *et al*., 2008) accompanied with diseases mainly bacterial wilt and late blight (Muthoni *et al.*, 2013) and soil acidity (Muthoni, 2016) contribute to low potato production. Low soil fertility is mainly caused by continuous cultivation without adequate replenishment of required amount of mined nutrients. Potato is a heavy feeder thus require a variety of elements for growth and development of which Nitrogen (N), Phosphorus (P) and Potassium (K) are among the most important elements (Pervez *et al*, 2013). Apart from N, P and K as major nutrients, potato require other secondary macronutrients such as Magnesium (40-60kg ha^{-1}) and calcium (100-120 kg ha^{-1}) as reported by Haifa (2020).

Currently there are different types of NPK fertilizers with additional nutrients including micro- nutrients available in Kenya. However, lack of information on the benefits of new fertilizer formulations, has limited their utilization. Other factors limiting the use of such fertilizers include access and availability. Calcium is a component of cell wall, it helps plants to resist stress condition and plant pathogens attack whereas magnesium, has a crucial role in photosynthesis, and is involved in production of sugars and proteins (YARA, 2020).

Furthermore, diseases are another major constraint that limit potato production in Kenya. Bacterial wilt caused by *R. solanacearum* is one of the most serious potato disease affecting over 70% of potato farms, causing yield losses of between 50% to 100% and has spread to all potato growing areas in Kenya (Muthoni *et al*., 2014a). *Ralstonia solanacearum* has a potential to survive for 3-4 years even under fallow or non-host plant, which make it very difficult to manage. Surface runoff, infested seeds, improper crop rotation and absence of effective control methods are the main factors that enhance the spread of this disease to most of the potato growing areas (Janssens *et al*., 2013).

Biofumigation including use of brassica crops (Canola, Mustard, etc.) as one of environmentally friendly methods of managing plant pathogens has been reported to have a potential in management of soil borne diseases, nematode and weeds (Larkin & Griffin, 2007; Rick *et al*., 1995). These plants produce glucosinolates that undergo hydrolysis to make isothiocyanates in the presence of enzymes myrosinase and moisture (Kirkegard, 1998). Larkin and Griffin (2007) evaluated the effect of brassica crops, including canola, rapeseed, radish, turnip, yellow mustard, and Indian mustard on potato soil borne pathogens. Results showed that the growth of (*Rhizoctonia solani, Phytophthora erythroseptica, Pythium* *ultimum, Sclerotinia sclerotiorum,* and *Fusarium sambucinam)* were inhibited by barassica crops, with the highest inhibition of (80 to 100%) resulting from Indian mustard application under *in-vitro* experiment. In the same study, under field experiment, Indian mustard was most effective for reducing powdery scab and common scab diseases, whereas rapeseed and canola were most effective in reducing *Rhizoctonia* diseases. Fulya *et al.* (2020) found that green manure of yellow mustard (*Sinapis alba*), turnip (*Brassica rapa*), arugula (*Eruca vesicaria*), Mighty mustard (*B. juncea*), rape (*B. napus,*), mustard green (*B. carinata*) and brown mustard (*B. juncea*) cover crops were effective in suppressing *Rhizoctonia solani* and *Phytophthora nicotianae.* Therefore, there is a gap on the use of brassica plants (Canola) on bacterial diseases management including potato bacterial wilt as one of the soil borne diseases.

1.2 Statement of the problem

Potato (*Solanum tuberosum* L.) yield in Kenya is far below the potential and there has been a production decline since 2010, while the demand for potato is increasing. Bacterial wilt and poor soil fertility management are some of the challenges that cause reduction in potato production. Long-term dependence of N- P based fertilizers that do not replenish all nutrients mined by potato. This has created secondary macro and micronutrients deficiencies in potato growing areas while potato require high amount of K, Ca and Mg for better production. Even though potato requires these elements, its production in Kenya is still done by most of farmers without this consideration due to previous unavailability of compound fertilizers in the market. Bacterial wilt caused by *R. solanacearum* is another serious problem facing potato production in Kenya as it has spread all over the country, affecting over 70% of potato farms and can cause yield loss ranging between 50-100%, and remains in the soil for 3-4 years yet the available means of management are not efficient. Canola green manure has the potential to suppress soil pathogens yet its effect on *R. solanacearum* is not well understood. There is a need for sustainable way of managing bacterial wilt and soil fertility in potato producing areas.

1.3 Objectives

1.3.1 Broad objective

To contribute to food security through enhanced potato *(Solanum tuberosum)* production in Kenya by addressing nutrients deficiencies and use of canola green manure for the management of bacterial wilt.

1.3.2 Specific objectives

i. To determine the effect of canola extract on *Ralstonia solanacearum* population density.

ii. To determine the effect of canola green manure and compound fertilizer application on bacterial wilt incidence in potato varieties.

iii. To determine the effect of canola green manure and compound fertilizer application on growth, nutrient use efficiency and potato yield for selected varieties.

1.4 Hypotheses

i. There is no significant effect of canola extract on *Ralstonia solanacearum* population density.

ii. There is no significant effect of canola green manure and compound fertilizer application on bacterial wilt incidence in potato varieties**.**

iii. There is no significant effect of canola green manure and compound fertilizer application on growth, nutrient use efficiency and potato yield for selected varieties.

1.5 Justification of the study

Potato is an important food crop and source of income in Kenya. It is grown by more than 800,000 farmers generating more than 500 million USD annually to the country (AGRA, 2019). However, this sector is experiencing production decline; soil fertility decline and bacterial wilt have been reported as main contributing factors. Long-term dependence on N, P-based fertilizers mostly DAP has been reported to contribute to gradual soil acidification and micronutrient deficiencies (Muthoni, 2016). Acidic soil affect availability and uptake of most essential nutrients by plant hence leads to poor growth and yield performance. There is a need for sustainable way of soil fertility and bacterial wilt management. In this study, canola green manure was used for the main purpose of managing potato bacterial wilt. Brassica family crops including canola has been shown a potential to inhibit soil borne pathogens through release of glucosinolate compounds. In addition, green manures also are known to supply and enhance nutrients availability through improvement of soil physical, biological and chemical properties. (NPK+Ca+Mg+) fertilizer is used as a balanced nutrients source and it supply most of essential nutrients required by potato growth and yield. This fertilizer maintains soil pH and does not have a side effect of soil acidification like DAP.

CHAPTER TWO

LITERATURE REVIEW

2.1 Potato production in Kenya

Potato (*Solanum tuberosum.*L) was first domesticated in southern Peru and northwestern Bolivia and it has become worldwide staple food for many countries (Hijmans & spooner, 2001). Worldwide it is the fourth after rice, wheat and maize (FAO, 2008b). In 2018, worldwide production was around 368 million tons and about one third of the total world potato production are produced in China and India (Statista, 2020). In Kenya potato was introduced first by European settler farmers during the late 19th century in Kiambu, Murang'a and Nyeri districts. Around 1920, the indigenous Kenyan farmers started potato production using Kerr's Pink as a main variety (MOALF, 2016a). Around 1967, Kenyan Government started a potato development project, with the main purposes of potato variety screening, plant breeding, seed multiplication, and agronomy (Theisen, 2006). Total potato production in Kenya has been fluctuating, even though the production area was increasing (FAOSTAT, 2020).

2.2 Importance of potato production in Kenya

Potato (*Solanum tuberosum.*L) in Kenya is an important food and cash crop that plays a major role in food security and is second to maize in terms of utilization. Potato demand has been increasing due to increased consumers and processors (Abong *et al.*,2011).There are approximately 800,000 potato growers in Kenya (Kaguongo *et al*. 2008) with an annual potato production of 2-3 million tons of potatoes. Annually 40-50 billion are produced and engaging millions of Kenyans (AGRA, 2019; Machangi *et al*., 2016). In Kenya 90% are small-scale farmers holding less than one hectare of farm whereas less than 0.05% of potato growers have more than 25 ha of land (Janssens *et al*., 2013). In Kenya as in the Sub-Saharan Africa (SSA) countries, roots or tuber crops are a major source of sustenance, especially potato accounts for more than 20 % of calories consumed world over. Potato production and processing gives employment to thousands of Kenyans. Besides general home use, potato is processed into may products like chips (French fries), crisps and frozen fries liked by many peoples and this increased potato demand and growth of fast food restaurants and snack bars in the urban areas (Abong *et al*., 2011).

2.3 Soil and ecological requirements for potato production in Kenya

In Kenya there are 13 major potato-producing counties; Meru, Nyeri, Nyandarua, Kiambu, Taita-Taveta. Nakuru, Narok, Bomet, Elgeyo-Marakwet, Trans-Zoia, Bungoma, Uasin-Sishu and Pokot. These areas are mainly located in Central, Rift Valley and Eastern provinces of Kenya. Potato grows well in cool climate mostly in high land at altitude ranging between 1500-3000 m.a.s.l. It grows well between 15℃ to 24℃ mean daily temperature with 1200-1800 annual mean rainfall (NPCK, 2019). Potatoes perform well in loose loamy soils (sandy and silt loam), well drained, rich in organic matter with a pH range of 5.0-6.5. Impermeable or hardpan soil layers limit the rooting depth system thus affecting potato production (Greenlife, 2020). The desirable saturation range of Ca and Mg on CEC are 60-70% and 15-20% respectively (Steyn & Plessis, 2020). Potato requires high soil moisture content for better production; therefore, it is recommended to maintain moisture above 70% of field capacity in the top soil 60cm (Foround *et al*., 1993).

2.4 Potato production constraints in Kenya

Potato production in Kenya has been facing so many constraints affecting the whole value chain. Limited use of certified/clean seed potato being a leading production constraint in Kenya. Most of potato farmers 96.3% uses informal seed potato while around 3.7% uses quality seed, which comprises certified seed, clean seed and positively selected seed (Kaguongo *et al*., 2009). Informal seed sources that include farm-saved (self-supply), local markets or neighbors are on the top of enhancing the spread of diseases including bacterial wilt. Diseases are another potato production constraint and bacterial wilt is the most prevalent disease, affecting 77 % of potato farmers, followed by late blight (67 %) and viral diseases (12 %) (Muthoni *et al*., 2013). Low soil fertility is another production constraint mainly due to continuous cropping without replenishing all mined nutrients. Potato production in Kenya is mostly occupied by smallholder farmers, with a land size below 5 acres (Okello *et al*., 2016) leading to intensively land use without fallow. In major potato growing areas in Kenya, the soil phosphorus is as low as 2.9 ppm while total nitrogen is lower than 0.15%. In addition, the available fertilizer recommendations do not consider for potassium (K) yet some studies have indicated the benefit of potassium addition in potato production (Muthoni & Nyamongo**,** 2009). Poor market structure and poor storage facilities is another problem facing potato producers in Kenya leading to low net return from potato production. Most farmers depend on rain for production, this leads to high production during rainy season. Moreover, most farmers do not have proper stores, which makes them selling directly from the field to the brokers at lower prices (Muthoni & Nyamongo**,** 2009).

2.5 Fertilizer use in potato production in Kenya

Low soil fertility is one of the factors contributing to low potato yield in most parts of the world (Nityamanjari, 2018). Fertilizer application is one of the best means of increasing yield per unit area (Ali & Iqbal, 2011). Kenya ranks 78th in fertilizer use globally; in sub-Saharan Africa (SSA) the country comes second after South Africa with average national fertilizer rates of (31.3 kg ha^{-1}) which is much higher than the average (9 kg ha^{-1}) for SSA. However, this is still below the internationally recommended standards of 50 kg ha^{-1} (Muthoni, 2016).

The most commonly used fertilizers in Kenya are DAP, Monoammonium Phosphate (MAP), Triple super Phosphate (TSP), Single Super Phosphate (SSP), NPK 20:20:0 and NPK 23:23:0 for planting (basal application) and Calcium Ammonium phosphate (CAN), Ammonium Sulfate Phosphate (ASN), Urea and Sulfate Ammonium (SA) for top dressing. These two groups account for 48.56% and 25.36% of the national fertilizer consumption, respectively (Muthoni, 2016). In potato production, DAP is mostly used at 96%, NPK at 1% and CAN at 8% (Kaguongo *et al*., 2008). The most widely used fertilizers in Kenya since the 1960s contained nitrogen (N) and phosphorus (P) due to assumption that most Kenyan soils have adequate supplies of other essential nutrients (Muthoni, 2016). It recommended to apply DAP at the rate of 500kg ha^{-1} during planting and 300 CAN as top dressing (NPCK, 2013) but most of potato farmer in Nakuru apply less than that (Ogola *et al*., 2011).

2.6 Potato nutrients use efficiency (NUE)

Potato (*Solanum tuberosum* L.) is a short duration crop, nutrient exhaustive and short duration crop that needs higher quantities of fertilizers and pesticides to produce high yield as compared to other crops. Among the plant nutrients, potato requires a variety of elements for growth and development of which N, P, K, Ca and Mg are the most important elements (Haifa, 2019; Westermann, 2005). Potato crop feeds heavily on soil potassium and the tubers remove 1 to 5 times the amount of nitrogen and 4 to 5 times the amount of phosphate in addition potato also acts as an indicator crop for K availability because of its high K requirement (Mikkelsen. 2006; Nityamanjari, 2018). Nutrient uptake is at its peak during tuber bulking (intensive volume increase process) (Haifa, 2020). Depending on the soil type, variety, crop rotation, moisture supply and management practices, potato (38.5 t ha^{-1}) can remove over 200 kg ha^{-1} of potassium and 120 kg $ha^{-1}$of nitrogen, both potassium and nitrogen are needed throughout vegetative growth, tuber formation and bulking (YARA, 2019). Koch *et al.* (2019) reported that one ton of potato could remove 3, 1.2, 5.2, 0.2 and 0.3 of nitrogen (N), P_2O_5, K_2O, MgO and CaO respectively (Figure 2.2).

The most important macronutrients are nitrogen (N), phosphorus (P), potassium (K). Sulphur (S), calcium (Ca) and magnesium (Mg), while micronutrients include iron (Fe), boron (Bo), zinc (Zn), manganese (Mn), copper (Cu), molybdenum (Mo), chlorine (Cl) and nickel (Ni). **Nitrogen** plays a key role vegetative growth of plants (crop canopy) with adequate level of the nutrients like phosphorus and potassium. It is an essential component of chlorophyll, amino acids and other plant building blocks, which are necessary for photosynthesis to take place. It is also required for cell division, composition of vitamins and carbohydrates (NPCK, 2018)**. Phosphorus (P)** is an essential element in every metabolic process and is required during early plant growth for proper early root formation and tuber initiation and at the end of the growth cycle, to promote tuber maturity. The primary role of phosphorus is to facilitate the transfer of sugars synthesized in the leaves to their storage site in the tubers and their interconversion to sugars and starch. At tuber initiation, phosphorus stimulates formation of many tubers but plays only an indirect role in tuber enlargement. A large number of tubers per plant are a desirable trait in crops grown for seed. P also helps the plant to survive harsh cold conditions and enhances efficient use of water (NPCK, 2018). **Potassium** has various roles in photosynthesis process, K regulates the opening and closing of stomata, and thereby regulates Carbon dioxide CO2 uptake. K plays a related role through the regulation of water loss in plants (known as osmo-regulation). It regulates both water uptake through the potato plant roots, water circulation within the plant and its subsequent loss from the leaves through the stomata. Furthermore, K has been shown to improve drought resistance. K is essential for both protein, starch synthesis, and is important in metabolism, breakdown and distribution of sugars within plant tissues (NPCK, 2018).

Fertilizers either organic or inorganic is one of expensive input applied by farmers to boost production and the only way to recover that cost is to increase nutrients use efficiency (NUE). Nutrients use efficiency is controlled by numerous factors and the main ones are; genetic nature of plants, physiological factors, environmental variables and plant interactions. Worldwide it is estimated that fertilizer application use efficiency to be about or lower than 50% for N, less than 10% for P, and about 40% for K, due to many factors (Baligar *et al.*, 2001). There are several ways nutrients can be lost or become unavailable which include; leaching, run-off, gaseous emission and fixation. Furthermore, soil salinity and/ or soil acidity can affect NUE. The physical factors can influence low NUE such as high bulk density, hard pans, poor soil structure and texture, surface sealing and crusting, high or low water holding capacity, water logging and extreme drying or poor aeration (Baligar *et al*., 2001).

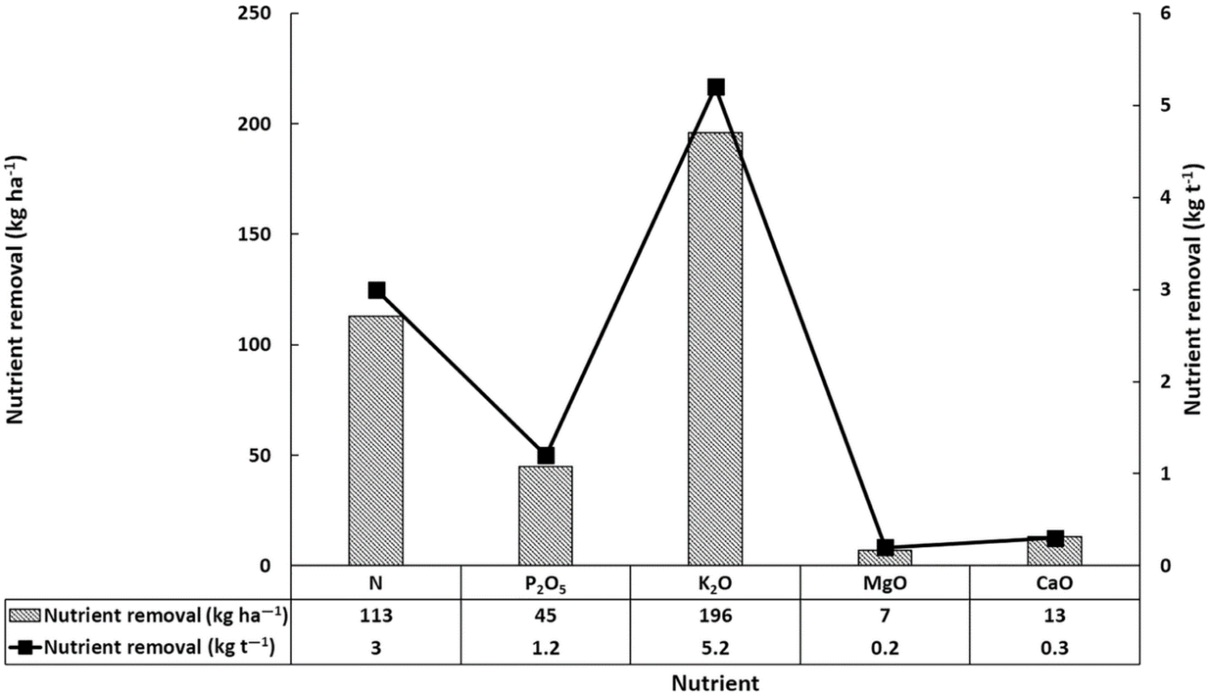


Figure 2.1: Removal of Nitrogen, Potassium, Phosphorus, Magnesium and Calcium by potato tubers (Koch *et al*., 2019)

Most of potato production farms in Kenya have become acidic, and this is reported to be a result of continuous application of ammonium-based fertilizers mostly DAP (Muthoni, 2016) and acidic soils can lead to low NUE. Adding phosphorus to a low pH soil renders it unavailable through fixation by Fe and Al oxides. However, the potato has a shallow rooting system to exploit soil nutrients fully such as (phosphorus and Nitrogen) hence; they are susceptible to losses through immobilization, volatilization, leaching and runoff under poor agronomic management (Hopkins *et al*., 2014; Rens *et al*., 2018).

Nutrient use efficiency can be improved through use of best management practices including applying nutrients at the right rate, right time and in the right place (Robert, 2008). Some nutrients like (N) are prone to leaching, split applications of N during the growing season, can enhance nitrogen use efficiency (Cassman *et al*., 2002). Method of fertilizer application is a factor to consider when applying fertilizer because it may affect NUE. Adrien (2008) reported that potato crop performed better with split fertilizer application than single application. There are several methods of fertilizer application including broadcasting and localized application. Robert (2008) reported that, nutrient recovery efficiency tends to be higher with band application and may be because of reduced nutrient loss due to leaching or fixation reactions.

2.7 Nutrient use efficiency assessment

In crop production, different inputs are used and can produce a range of outputs. The overall efficiency is assessed based on output. Maximum profit occurs when the maximum value of outputs is reached for each unit value of all inputs. At the rate where the net return to the use of one input peaks, the input is making its maximum contribution to increasing the efficiency of all other inputs involved. There are several assessments of nutrient use efficiency, where four are commonly used (Dobermann, 2007) (Table 2.1).

Table 2.1: Common nutrients use efficiency terms and their application.

Term	Calculation	Question addressed	Typical use
Partial factor productivity	PFP = Y/F	How productive is this cropping system in comparison to its nutrient input?	As a long-term indicator of trends.
Agronomic efficiency	AE = (Y-Y0)/F	How much productivity improvement was gained by use of nutrient input?	As a short-term indicator of the impact of applied nutrients on productivity. Also used as input data for nutrient recommendations based on omission plot yields.
Partial nutrient balance	PNB = UH/F	How much nutrient is being taken out of the system in relation to how much is applied?	As a long-term indicator of trends; most useful when combined with soil fertility information
Apparent recovery efficiency by difference	RE = (U-U0)/F	How much of the nutrient applied did the plant take up?	As an indicator of the potential for nutrient loss from the cropping system and to access the efficiency of management practices.

* Y = yield of harvested portion of crop with nutrient applied; Y0 = yield with no nutrient applied; F = amount of nutrient applied; UH = nutrient content of harvested portion of the crop; U = total nutrient uptake in aboveground crop biomass with nutrient applied; U0 = nutrient uptake in aboveground crop biomass with no nutrient applied.

2.8 Potato growth and yield parameters as influenced by soil amendments

Soil amendments are the source of plant nutrients. Potato require a variety of elements for growth and development of which Nitrogen (N), Phosphorus (P), Potassium (K) Magnesium (Mg) and Calcium (Ca) are among the most important elements (Haifa, 2020; Pervez *et al.*, 2013). Nitrogen and potassium are removed in large quantities, both by tops and tubers (Mikkelsen, 2006). This means these two elements are most likely to be deficient in normal agricultural soils and must be supplied in adequate quantities to obtain higher yields (Nand *et al*., 2011). Growing healthy potatoes for maximum yield and quality requires that all the essential nutrients be supplied at the 4R principles (right source, right rate, the right time and the right place). In potato production, plant nutrient should be regulated not deficient or excessive because can reduce tuber bulking and quality. Nutrients deficient soils may limit the potato canopy growth, resulting in reduced carbohydrate accumulation and tuber growth. Maintaining healthy leaves is a key to producing high yields (Mikkelsen, 2006). Tsegaye (2017) reported that, excessive nutrients application may cause nutrient imbalances or stimulate over potato vegetative growth that may limit tuber production. Mostly, growth of potato is positively correlated to tuber yield. Fayera (2017) found that the tuber yield per hectare was positively correlated with plant height, number of stems per plant, number of tubers and weight of flesh tuber per plant. Tsegaye (2017) found that fresh tuber yield of potato was positively and strongly correlated with number of plants per hill, number of tubers per hill and fresh tuber weight per plant and total dry tuber yield of potato was positively and strongly correlated with total N, P, K uptake.

2.9 Potato bacterial wilt disease in Kenya

Bacterial wilt also known as brown rot is potato disease and is one of the most destructive diseases of potato, caused by the pathogen called *Ralstonia solanacearum*. CGIAR (2005) reported that is the second most damaging potato disease after late blight in tropical and sub-tropical areas. The pathogen is classified into different races and biovar and potato bacterial wilt is caused by race 3 biovar2. A Portuguese first described race 3 around 1947 and it was spread due to importation of potatoes form Mediterranean basin where it was first found. Around 1980s and 1990s, the disease was further spread in Europe by irrigation (CABI, 2020). In Kenya, the disease was imported from Europe and it was first reported in Embu area around 1945 where it was spread all over the countries (Muthoni *et al*., 2014).

The development of pathogen is favoured by tropical and sub-tropical environmental conditions. The temperatures between 24 to 35°C with optimum of 27°C and usually does not cause problems in areas where the mean soil temperature is below 15°C (Lemay, 2003). Bacterial wilt disease also affects over 200 plant species (EPPO, 2004), of which the most susceptible plants are potatoes, tomatoes, pepper, eggplant and groundnut.

Globally, the disease is estimated to damage over 950 million USD annually and about 1.7 million hectares of potato are affected by bacterial wilt disease in approximately 80 countries (Champoiseau, 2008a). It is a constraint for potato production mostly in Sub-Saharan Africa. Kenya is one of the most affected country in Africa and affecting over 70% of potato farms and causing yield losses of 50 to 100%. The speed of spread is associated with limited farmer's skills on disease management and prevention, and unavailability of enough healthy potato seeds (Muthoni *et al*, 2014).

2.10 Bacterial wilt disease cycle

Ralstonia solanacearum is soil borne and can be seed borne or water borne pathogen. *R.solanacearum* enters into plant through wound on root and enter through the xylem in the vascular bundles and colonize the plant (Champoiseau *et al*., 2009) and this leads to the obstruction of the vessels and the subsequent development of the typical wilting symptoms due to impaired water conductance (Hao *et al*., 2020). The pathogen can survive beneath the soil surface in vegetation free fields or inhabit in non-host plants without showing any disease symptoms (Kakuhenzire *et al*., 2013). The pathogen can survive for eight years or even longer in the soil, depending on factors such as the quantity of bacteria in the soil, soil type, temperature, water status, soil depth and the presence of host plants (Potatoes South Africa. 2015). The pathogen has may ways of spreading, it can spread through infested planting material, running water from infested field or infested irrigation water, infested crop debris, movement of infested soil and contaminated farm tools and equipment (Kinyua *et al*., 2014).

2.11 Bacterial wilt symptoms

Aerial part of infested plant in the early stages of disease, the first visible symptoms is wilting of the youngest leaves. At this stage only one or half a leaflet may wilt, this can appear during the hottest part of the day and plants may recover at night or early in the morning, when the temperatures are cooler (Champoiseau, 2008a). Under favorable conditions disease develop and all the leaves eventually wilt leading to plant death (Plate 2.3B) (Champoiseau, 2008a). Wilting caused by *Ralstonia solanacearum* may look like the one caused by insufficient of water, insects or mechanical damage if not well diagnosed (Martin & French, 1985). Another common symptom that can be associated with bacterial wilt disease in the field is stunting and yellowing of plants. A brown discoloration may be shown on cross-sections of stems and stolons severely infected. On tubers a cross-section of infected potato tubers may show a grey-brown discoloration of vascular tissues (called: vascular ring) and as infection progresses, the discoloration may extend into the pith or cortex of the tuber (Plate 2.3A). A milky-white sticky ooze, which indicates the presence of bacteria cells, might also be observed from freshly cut sections of infected tubers. Another common sign of bacterial wilt disease that can be observed at the surface of freshly cut sections from severely infected stems is a thread of bacterial slime ooze out of the vascular bundles, which indicates the presence of dense masses of bacterial cells in infected vascular bundles, and particularly into the xylem (CABI, 2020).

Plate 2.1: Symptoms of potato infested by bacterial wilt (A) on tuber and (B) on foliage).

Bacterial wilt management

The bacterium is very difficult to control because it has wide host range and wide biological variation (Martin & French, 1985). Several methods of control have been tried but no single method that gives total control, though some level of bacterial wilt disease management may be observed through the use of integrated methods. Planting of disease-free tuber seeds, rouging of infested plants, weeding and quarantine are ones of the disease management measures referred to as phytosanitary method (Champoiseau *et al*., 2010). The use of cultural methods including crop rotation, intercropping, delayed planting and soil amendments is a way of reducing bacterial incidence (Champoiseau *et al*., 2010; EPPO, 2004). Rotating potato with none hosts crops like maize, okra, cowpea and french marigolds in cropping system have been reported to reduce the incidence of bacterial wilt in infested potato (Saddler, 2005). Rotating potatoes with some none host vegetables like cabbage shown to reduce bacterial wilt disease at certain level. For better results, potatoes should be rotated with cabbages, beans, peas, onions, carrots, pasture grass or pasture legumes (The Organic Farmer, 2012). A number of soil fumigants like chloropicrin have been tested for bacterial wilt control in many crops; however, fumigation is not economically feasible over large areas and has environmental hazards (Muthoni *et al*., 2012).

Amending agricultural soils and soilless growing media with organic matter supplies plant nutrients, increases natural suppressiveness of the soil against soil-borne pathogens and improves physicochemical and biological characteristics (Janvier *et al.*, 2007). Incorporation of household compost, cow dung manure and pig slurry have been found to reduce bacterial wilt incidence and severity. For instance, soil amended with organic materials, inorganic materials (NPK fertilizers) or different combinations of these amendments considerably affected bacterial wilt incidence on potato and increased tuber yield (Lemaga *et al.*, 2005). Islam and Toyota (2004) reported suppression of bacterial wilt in soils amended with poultry manure and farmyard manure. Research conducted by Larkin *et al*. (2011) indicated that the use of disease-suppressive green manures.

2.12 Use of biofumigants

The use of green manures, which involves the incorporation of fresh plant material into soil, has been mostly for adding nutrients into the soil and improvement of soil health through soil physical, chemical and biological properties. However, green manures from certain plants (e.g., Canola and Mustard) has been found to have additional benefit of suppressing growth and development of soil pathogens, weeds and nematodes (McGuire, 2016). Green manure produces many changes in soil physical, chemical and biological properties, and may also increase soil microbial biomass and activity, and cause distinct changes in soil microbial populations that may be partially responsible for suppression of diseases (Larkin, 2013). However, green manures of different crops and cultivars may vary considerably in their activity or efficacy against different pathogens and diseases. In particular, green manures of brassica and related crops have been shown to release bio-toxic compounds or metabolic byproducts that exhibit broad activity against soil pathogen, insects, nematodes, and weeds (Clark, 2007).

Canola and all other members of the Brassicaceae family accumulate glucosinolates, a diverse group of sulfur containing compounds. The major crops in the Brassicaceae family used in bio-fumigation are broccoli, cauliflower, turnip, canola, cabbage, kale and radish (Larkin, 2013). Enzymatic hydrolysis of glucosinolates by thioglucoside glucohydrolases (myrosinases) results in the formation of isothiocyanates and these chemicals are similar to the active chemical in the commercial fumigant metham sodium (McGuire, 2016)**.** Brassica green manures have been used in management of a variety of plant pathogens. Although green manure provides partial disease reduction and can be variable. This method should be used as an important component within a larger integrated disease management program to provide improved sustainable production systems (Larkin, 2013).

CHAPTER THREE

EFFECT OF CANOLA EXTRACT ON *Ralstonia solanacearum* POPULATION DENSITY AND MORPHOLOGY

Abstract

Diseases are among the major challenges facing potato production in Kenya. Bacterial wilt caused by *Ralstonia solanacearum* is one of the key diseases affecting potato production. Biofumigation has been used as an eco-friendly method of managing plant pathogens. An experiment was conducted at Egerton university laboratory to evaluate the effect of canola extract on *R. solanacearum* population density and its morphology *in vitro*. Four levels of canola extract quantities were used; 0, 100, 200, 300 microliters, and enrich immunomodulator (Di-bromo Di-nitro propane 1-3-diol) (DDD) at the rate of 1ml/petridish was used as a positive control. Inoculum was isolated from infested soil on Selective Medium South Africa (SMSA) (containing; Casamino acids, Bacto-Peptone, Glycerol, and Bacto-Agar) and used for growth arranged in a completely randomized design. Bacterial population density in colony forming units (CFUs) and morphology data were taken after 3 days of incubation. The data showed that canola treatments did not have significant effect on CFUs at 5% level of significance. Canola extract seemed to enhance growth of bacteria over control. The cell shape and colony margin were not affected by canola extract treatments under microscope observation. Di-bromo Di-nitro propane 1-3-diol restricted the growth of *R. solanacearum*. Canola did not suppress the growth of *R. solanacearum in-vitro*; further investigation should be done on other brassica family crops.

3.1 Introduction

Ralstonia solanacearum is a pathogen that cause bacterial wilt and is ranked as second most important pathogen for potatoes (Yuliar & Toyota, 2015). *Ralstonia solanacearum* is soil borne, gram negative, rod shaped with 0.5-0.7 X 1.5-2.0 µm in cell size (Heather, 2005). The pathogen is classified in five races based on host ranges; race 1 (solanaceous vegetables), race 2 (banana), race 3 (potato and tomato), race 4 (ginger), and race 5 (mulberry) with six biovar based on ability to utilize disaccharides and to oxidize hexose alcohol (Liu *et al*., 2016). *Ralstonia solanacearum* enters into plant through wounds on roots. Such wounds may be caused by lateral root emergence or by root damage caused by soilborne organisms like nematodes. Stem injuries from insects, handling, or tools can also be the way of bacteria entry. Once bacteria infect the roots or stems, they enter through the xylem in the vascular bundles and colonize the plant (Champoiseau *et al*., 2009). The pathogen can survive beneath the soil surface in vegetation free fields or in non-host plants without showing any disease symptoms (Kakuhenzire *et al*., 2013). It is an important potato pathogen causing bacterial wilt worldwide with high yield losses. It is estimated to affect more than 200 species, under solanaceae (potato, tomatoes, and tobacco), musaceae (banana), and fabaceae (groundnut) (Yuliar & Toyota, 2015). Wilting of plant, discoloration of vascular tissues on tubers are most common symptoms of the disease in potato.

The pathogen is difficult to control, because it has a wide host range, long survival in the soil, wide biological variation and no single method has been found to be effective alone (Karim *et al*., 2018). Brassica plants produce glucosinolates compounds that are hydrolysed into active products including isothiocyanates, thiocyanates and nitriles in the presence of endogenous enzyme (myrosinase) (Mithen, 2001). Due to their electrophilicity, glucosinolates and isothiocyanates in particular have antifungal, antimicrobial as well as insecticidal properties (Hanschen *et al*., 2017). These products, causes degradation of enzymes and the inhibition of microbial growth through reaction with sulph-hydryl groups, amine groups and the disulphide bonds of proteins (Mithen, 2001). Due to this toxicity, brassica family crops can be used in management of plant pathogens, though the most available literatures focus on fungal pathogens. Therefore, this study was carried out to determine the effect of canola extract on *R. solanacearum* population *in-vitro*.

3.2 Materials and methods

3.2.1 Experimental procedure

Laboratory experiment was conducted at Egerton University biological science laboratory. Canola (*Brassica napus* L.) was grown at Egerton University field (7) for two months. At early flowering stage; the fresh leaves, stems and shoots (20 g) were collected, washed in water and surface-sterilized by dipping them in 1% sodium hypochlorite for 1 minute followed by three rinses in sterile distilled water and dried on absorbent filter paper. The leaves were chopped and thoroughly macerated with a mortar and pestle (Sintayehu *et al*., 2014). The extracts were obtained by squeezing (by hands) macerated products and then poured in a sealed bottle for use in subsequent experiments.

A bacteria inoculum was prepared from infested soil from Elburgon with the initial bacterial colony density range of 2-5 × 10^3 CFU/ml. Five grams of soil was weighed into a flask, then 50 ml of distilled water added. The mixture was shaken vigorously for 15 mins and allowed to settle for 5 mins. A bacterial suspension was taken to prepare two serial dilutions. From dilution two, (1 ml) of bacterial suspension was inoculated into each petridish. Zero, one hundred, two hundred and three hundred microliters of canola extract were poured into petridishes using micropipette. Enrich immunomodulator (Di-bromo Di-nitro propane 1-3-diol) (DDD) was used as a positive control (0.5 g was diluted in 1 ml of water and poured into petridishes. The quantity of canola extract used was based on 0.2 ml of cabbage (*Brassica oleracea*), Ethiopia mustard (*B. carinata*), rapeseed (*B. napus*), and garden cress (*Lepidium sativum*) extract used by Sintayehu *et al.* (2013). A specific medium for *R. solanacearum* “Selective Medium South Africa (SMSA)” composed of; Casamino acids, Bacto-Peptone, Glycerol, Bacto-Agar Crystal violet, Polymyxin ß sulfate, Bacitracin, Chloromycetin and Penicillin was prepared following Champoiseau (2008b) and Kinyua *et al*. (2014) and method. The medium at 40-45 ℃ was poured in each petridish containing (inoculum and canola extracts), mixed vigorously and allowed to solidify. Bacterial cultures were transferred to incubator for 72 h at 28 ℃. The experiment was laid down in a completely randomized design and replicated three times.

3.2.2 Data collection and analysis

Three days after incubation, number of colonies were counted in each treatment. For cell shape, crystal violet dye was used to stain the bacterial cells and visualized using light compound microscope (at 400× magnification). For bacterial colony margin visualization, Leica zoom stereo-microscope (at 300× magnification) was used. For analysis of bacterial colony density, General linear model (GLM) procedures of SAS (9.3) were used for ANOVA at $P \leq 0.05$. The treatment means were separated using Tukey’s honestly significant difference (HSD) test at 5% level of significance (Haynes, 3013).

Statistical model:

$$Y_{ij} = \mu + T_i + \varepsilon_{ij}$$

where:

Y_{ijkl} = Overall observations

μ = Overall mean

T_i = Effect due to i^{th} treatment

ε_{ij} = Random error

3.3 Results

3.3.1 Effect of canola extract on *Ralstonia solanacearum* colony density and growth

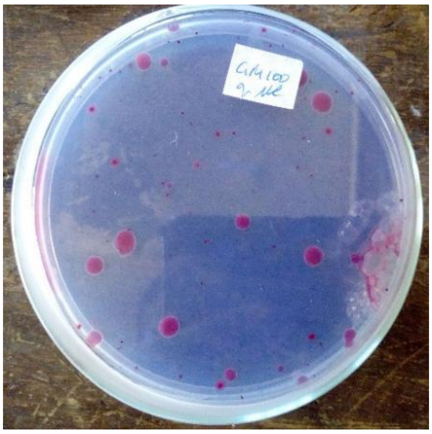
The result showed that, there was a significant difference among the treatments though, the three levels (100, 200 and 300 µl) of canola extract had no effect on *R. solanacearum* colony density. Negative control (no canola extract) exhibited slightly lower level of *R. solanacearum* colony density compared to canola extract treatments. The positive control treated with chemical immunomodulator (Di-bromo Di-nitro propane 1-3-diol) (DDD) had zero colony forming units (CFU) (Table 3.1). Dennett test showed that, negative control was significantly different from the 300 microliters canola extract treatment. After 48 hours of incubation, all bacterial colonies appeared in all petridishes (excluding positive control), but after 72 hours incubation, canola extract treatments seemed to enhance the growth of bacterial colonies (plate 3.1).

Table 3.1: Effect of canola extract on *Ralstonia solanacearum* density in-vitro experiment

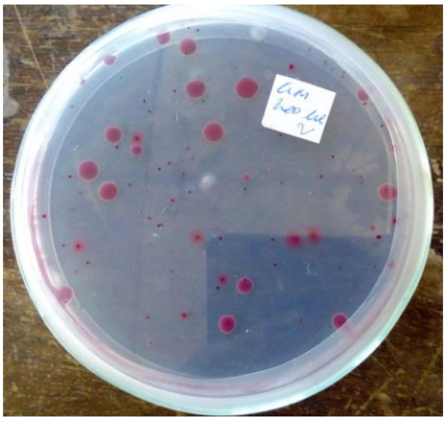
Canola extract	Means (CFU/ml) $\times 10^2$
300 (µl)	59.33^{a}
200 (µl)	28.00^{abc}
100 (µl)	33.67^{ab}
0 (µl)	20.00^{bc}
Chem (DDD)	0.00^{c}
MSD	32.26

The means followed by the same letters in the same column are not significantly different using Tukey's honestly significant difference (HSD) test at P <0.05. *CFU:* Colony forming unit, *MSD:* minimum significant difference. *Chem (DDD)*: Di-bromo Di-nitro propane 1-3-diol.

Chemical (DDD) **0 (µl) canola extract** **100 (µl) canola extract**



200 (µl) canola extract **300 (µl) canola extract**



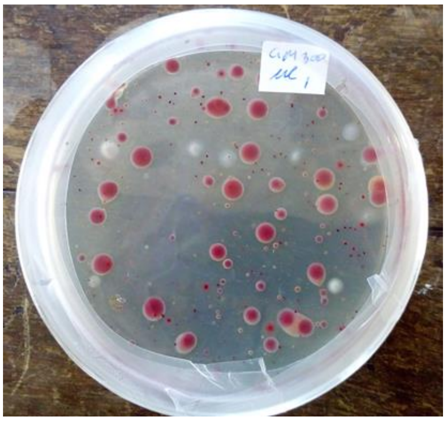


Plate 3.1: Effect of canola extract on *Ralstonia solanacearum* colony density at 72 hours of incubation. Chem (DDD): Di-bromo Di-nitro propane 1-3-diol.

3.3.2 Effect of canola extract on colony margin and cell shape

A microscope was used to visualize the margin of colonies as affected by canola extracts, plate 3.2 shows that there was no effect of canola extract on colony margin. The shape of cell was visualized also using a microscope, though no differences observed among the treatments.

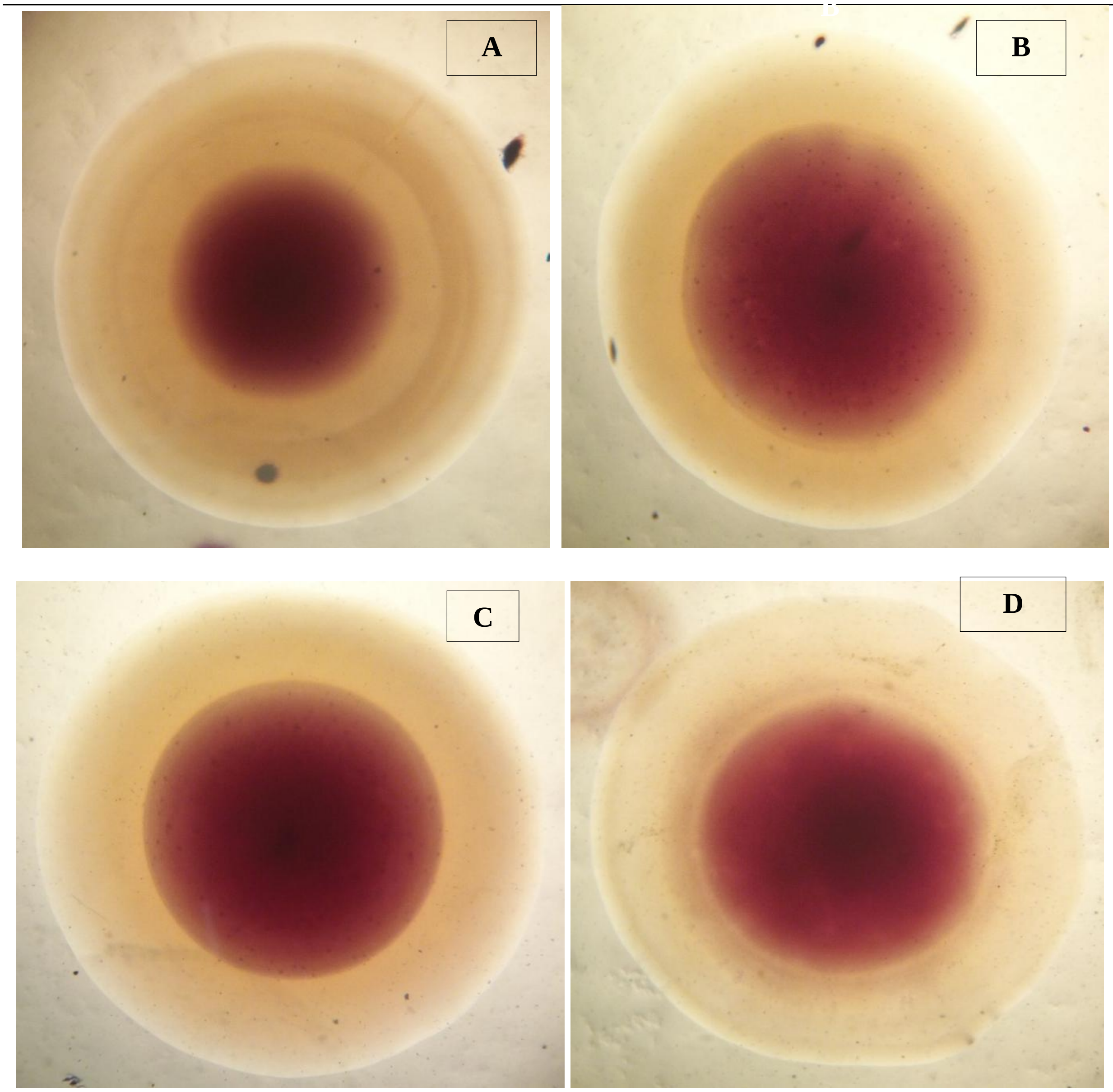


Plate 3.2: Effect of canola extract on *Ralstonia solanacearum* colony margin at 72 hours of incubation (400× magnification). A, B, C and D are 0 **µl**, 100 **µl**, 200 **µl**, and 300 **µl** of canola extract respectively.

3.4 Discussion

3.4.1 Bacterial colonies population density and growth as affected by canola extract

The growth of *Ralstonia solanacearum* bacteria on SMSA growth medium occurs in 2-5 days at 28℃ of incubation (Champoiseau, 2008b). Bacterial growth on plate is controlled by medium components and Agar concertation, and if agar remain, constant and nutrients increases may enhance growth of bacteria (Lacasta *et al*., 1999). Bacto peptone, Casamino acid and glycerol are reagents used to culture bacteria and are the main source of nutrients to the bacteria. In this study, the bacteria seemed to grow better in treatments where canola extract was added. This may be due to additional nutrients from canola extract. Moreover, canola extract did not show a biofumigation potential in suppressing *Ralstonia solanacearum* growth; this may be associated with low glucosinolate contents. Larkin *et al*. (2018) reported that canola has low, rapeseed, yellow mustard and oilseed radish have moderate while mustard duchess, mustard blend and oriental mustard have high glucosinolate content. Oplinger *et al*. (1989) reported that canola contains less than 3 mg/g of glucosinolates and this has achieved through breeding program. However, different authors found some positive effect of brassica against soil borne pathogens. Larkin and Griffin (2007) found that, the macerated leaf tissue from Indian mustard exhibited total inhibition of fungal pathogens (*R. solani, Phytophthora erythroseptica,* and *Pythium ultimum*) while canola resulted in lower inhibition under *in-vitro.* Sintayehu *et al.* (2014) reported that Ethiopian mustard and rapeseed extracts showed higher inhibition of *Fusarium oxysporum* growth compared to control *in-vitro*- experiment. Several types of plant extracts have been tested for their efficacy in management of *R. solanacearum*. Chen *et al*. (2016) evaluated plant derived *resveratrol* and *coumarin* concentration on *R. Solanacearum* in tobacco, found that, increasing concentration of phytochemicals significantly decreased growth rate of the bacteria. Li *et al*. (2016) on the study that was evaluating antibacterial and mechanism of action of plant-derived chemicals against *R. solanacearum* observed that Dimethylsulfoxide (DMSO) had no effect on the growth of *R. solanacearum* but protocatechualdehyde was able to inhibit *R. solanacearum* completely at concentrations of 40 μg/mL on agar media.

3.4.2 Colony margin and cell shape as affected by canola extract

The result showed that canola extract had no effect on *R. solanacearum* colony margin. The colonies were red/pinkish red at the center and whitish periphery with fluidal and irregular in shape as supported by Kinyua *et al*. (2014). Normally, the bacterial colonies are circular in shape with smooth margin. Jayesh *et al*. (2014) reported that *R. solanacearum* colonies on SMSA nutrient agar medium were smooth circular, raised and dirty white. Champoiseau, (2008b) reported that, on solid medium SMSA, bacterial colonies appear fluidal, irregular in shape, and white with pink centers and may appear 2-5 days after incubation at 28ºC. The results indicated that the colonies had pinkish red in centers and whitish periphery with irregular margins. Lacasta *et al.* (1999) reported that, bacterial colony can exhibit a great diversity of forms depending on culture conditions (agar concentration and nutrient availability). There are no early researches on the effect of canola extract on bacterial colony margin available, but some other plant derived extracts have been tested on their effect on *R. solanacearum. In-vitro* inhibition assay done by Deberdt *et al.* (2012) to evaluate the effect of three concentrations of *Allium fistulosum* extract on *Ralstonia solanacearum*, and inhibition of bacterial growth were observed, where strongest inhibition was observed at concentrations of 50% and 100%. Kumar *et al*. (2017) evaluated the efficacy of botanical plants extracts by the zone of inhibition assay technique against *Ralstonia solanacearum* causing bacterial wilt of tomato using water and ethanol extracts, undiluted *Ocimum gratissimum* extract showed highest inhibition zone of 28.66 mm.

Bacterial cell shape is genetically determined and primarily dictated by a polymeric macromolecular structure that surrounds the cytoplasmic membrane called peptidoglycan (PG) sacculus. Bacterial cell shape remains unchanged though, throughout the cell division variations may occur, and mostly influenced by environmental conditions (nutritional condition and stress response) (Van *et al*., 2017). In this experiment, the results showed that canola extract did not affect bacterial cell shape. The cells were rod shaped without any deformation. Kinyua *et al*. (2014) and Jayesh (2014) reported that *Ralstonia solanacearum* cells are gram negative, small straight rod shaped. Even though, there are limited information of brassica's effect on bacterial cell shape, other plant-derived products have been studied. Chen *et al*. (2016) in the study that was evaluating plant derived *resveratrol* and *coumarin* concentration on *Ralstonia solanacearum in* tobacco, observed effect of phytochemical on bacterial cell. With control, the cells were intact and uniform in size, with a clear boundary and a smooth surface but when treated with high concentration of phytochemical the cell appeared incomplete and turned flat in a dissolved shape, with an unclear boundary that was partially ruptured. Li *et al.* (2016) on the study that was evaluating the effects of the antibacterial and mechanism of action of Protocatechualdehyde against *Ralstonia solanacearum,* found that Protocatechualdehyde (PCA) treated bacteria, clearly destroyed the surface structure of the bacterial cells and shapes.

3.5 Conclusion

Brassica biofumigation has shown a potential in management of soil borne pathogens, though the potential differs from species to species. In this study, canola extract as one of the brassica crops did not show any effect on inhibition of *Ralstonia solanacearum* growth *in-vitro*. The study recommends further investigation on the effect other brassica plants.

CHAPTER FOUR
MANAGEMENT OF BACTERIAL WILT IN POTATO USING CANOLA GREEN MANURE AND COMPOUND FERTILIZER

Abstract

Potato bacterial wilt disease is a problem that is affecting potato production in Kenya. Two field trials and semi-field (pot) experiments were conducted to evaluate the effect of canola green manure and compound fertilizer on bacterial wilt management. Field experiment was carried out in two sites (Elburgon and Mau-Narok) while semi-field was carried out at Egerton university farm. Under field, two levels of canola green manure (with and without green manure application) and five levels of fertilizer were used in split-spilt plot design replicated three times. While under semi-field experiment, four levels of canola green manure (100, 75, 50 and 0 g kg^{-1} soil) and five levels of the compound fertilizer as used in the field experiment were used in a completely randomized design (CRD) with three replicates. Under field and semi-field experiments, canola green manure and fertilizer treatments had no effect on bacterial population density in the soil nor bacterial wilt incidence. Significant reduction of bacterial population density in the soil of 89.0%, 55.7% and 90.7% at Mau-Narok, Elburgon and Egerton sites respectively were observed at flowering stage as compared initial assessment. *Kenya Karibu* did not show any wilting symptoms caused by bacterial wilt across the sites and semi-field experiment. Further research should be done on other locally available plants of brassica family by determining those with high glucosinolates contents and their potential to control *R. solanacearum* and further screening for resistance of other varieties available in Kenya should be done.

4.1 Introduction

Potato bacterial wilt disease caused by *R. Solanacearum* is the second most destructive disease in Kenya after late blight that has spread to over 70% of total potato production farms in Kenya causing a yield loss of 50-100% (Muthoni *et al*., 2014). The bacteria appear in three races and five biovar, race 3, biovar 2 being important for *solanacea* family including potato (Monther & Kamaruzaman, 2010; Muthoni *et al*., 2012). The pathogen is soil borne, and can spread through infested potato seeds, infested running water, farm equipments, crops residues and infested shoes (NPCK, 2013). The bacteria infest plant from soil through wound that may be created by nematode, mechanical damage and/ or insect damage on root cortex and colonize the whole plant through xylem vessels, this leads to the obstruction of the vessels and the subsequent development of the typical wilting symptoms due to impaired water conductance (Hao *et al*., 2020). The bacteria can persist in soil for a long period up to five years (Stander *et al*., 2003) and the population increases easily with the presence of host plants (Arwiyanto *et al*., 2011). Wilting, yellowing and stunted growth are common symptoms, at early infestation, wilting mostly appear on young parts of plants but later the whole plant wilts and eventually dries (Agriculture, 2020).

Several measures have been used in management of this disease, including chemical (Yuliar & Toyota, 2015) cultural and soil solarization methods (Vinh *et al.*, 2005), however the disease seems difficult to manage. Chemicals are hazardous to environment, and some are prohibited in some countries due to the risks associated to the pesticide operators, as well as aquatic organisms, birds, and bees (Karim *et al*., 2018). Emphasis should be taken on use of certified disease-free seed, quarantine measures on infected fields and farms, sufficient crop rotation, reliable and early detection of the pathogen, removal of volunteer plants and management of nematodes where present, avoidance of surface water for irrigation and control of weed hosts (CABI, 2020).

Brassica family plants have been shown to have biofumigation properties that can be used in management of soil borne diseases, nematode and weeds (Larkin & Griffin, 2007; Rick *et al*., 1995). These plants produce compounds called glucosinolates that are released from plants through rupturing of plants tissues. Glucosinolates undergo further process (hydrolysis) to make isothiocyanates in the presence of enzymes myrosinase and moisture. Glucosinolates are not toxic at this stage, the isothiocyanates are the ones known to have broad biocidal activity including insecticidal, nematicidal, fungicidal, antibiotic and phytotoxic effects (Kirkegard, 1998). Although, disease reduction associated with green manures can be variable and provides only partial control, it can be used in an integrated disease management program (Larkin, 2013).

There is limited information on the use of brassica plants on bacterial disease management including potato bacterial wilt. Different authors revealed some levels of inhibition on fungal pathogen and reduction of fungal disease severity and incidences due to brassica green manure application (Fulya *et al*., 2020; Larkin & Griffin, 2007; Motisi *et al*., 2009; Sintayehu *et al.*, 2014). Therefore, experiments were conducted to evaluate the effect of canola and compound fertilizer on potato bacterial wilt disease management.

4.2 Materials and methods

4.2.1 Experimental sites description

Field experiment was conducted at Mau Narok and Elburgon sites, while semi-field experiment and laboratory were conducted at Egerton University, both sites are located in Nakuru County, Kenya (Figure 4.1). Egerton university site lies between longitude 35° 35' E, latitude 0° 23' S, and at an altitude of 2238 m.a.s.l. Mau-Narok is located in Njoro sub-county at an altitude of 2,900 meters above the sea level (m.a.s.l) and lies between longitude 36°0'E and latitudes 0°36'S. The area receives an average annual rainfall of 1,200-1,900 mm. The minimum temperatures of 6-14°C and maximum of 22-26°C (Abigael *et al*., 2014). The soil is well drained, deep to very deep, very dark greyish brown, friable and smeary, clay loam, with thick humic topsoil (mollic andosols) (Jaetzold *et al*., 2007). Elburgon is located in Molo sub-county at an altitude of 2,200 m.a.s.l and lies between longitude 35° 41'E and latitudes 0°12'S. This area experiences mean annual rainfall of 1000-1400 mm and mean temperatures of 13.7 -20°C (Jaetzold *et al*., 2007). The soils of Elburgon are acidic, well drained, deep, dark reddish brown with a mollic A horizon, and classified as mollic Andosols (Onwonga *et al*., 2014). According to MoALF (2016b), Elburgon site is in Upper high land zone two (UH2) and Mau-Narok is in Upper high land zone one (UH1). The sites were selected because are suitable for potato production.

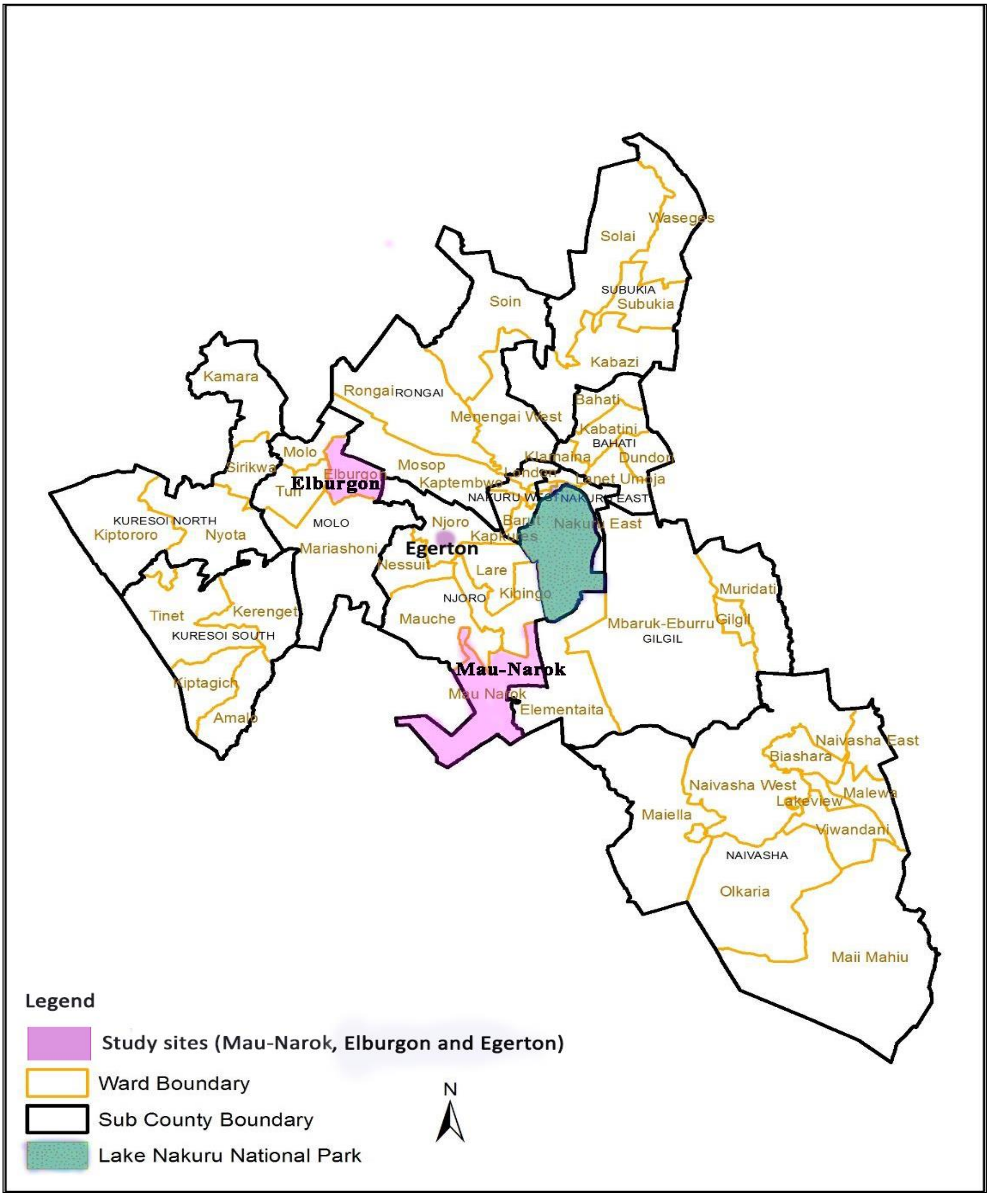


Figure 4.1: Map showing experimental sites Elburgon (longitude 35° 41’E and latitudes 0°12’S), Mau-Narok (longitude 36°0’E and latitudes 0°36’ S) and Egerton (longitude 35° 35’ E, latitude 0° 23’ S) (County government of Nakuru, 2018).

4.2.2 Germplasm description

Shangi and *Kenya Karibu* varieties were used in these experiments. *Shangi* is popular and the most grown variety in Nakuru. It is a semi- erect medium tall variety (slightly below 1 metre in height) with moderately strong stems and light green broad leaves, the flowers are pink in color (Plate 4.1A). It grows well in the altitude above 1500 m.a.s.l in the places like Nakuru, Bomet, Narok and Kericho. It matures early in about 3.5 months with the yield ranging between (30,000-40,000 kg ha^{-1}). The tubers are oval shaped, smooth cream skin, medium to deep eyes and white flesh (Plate 4.1B). It is moderately susceptible to late blight (NPCK, 2019). *Kenya Karibu* variety is one of the popular potato varieties, grows well at altitudes between 1800-2600 m.a.s.l in the place like Nakuru, Bomet, Narok, Nyandarua and Kericho. The variety is a high yielder (35,000 kg ha^{-1}- 45,000 kg ha^{-1}) and tolerant to late blight. The flowers are profusely light pink in color (Plate 4.1C). The tubers are round, smooth red skin, and yellow flesh with deep eyes (Plate 4.1D) (NPCK, 2019). The varieties were selected because are suitable for experimental sites and are grown by most of farmers in Nakuru.

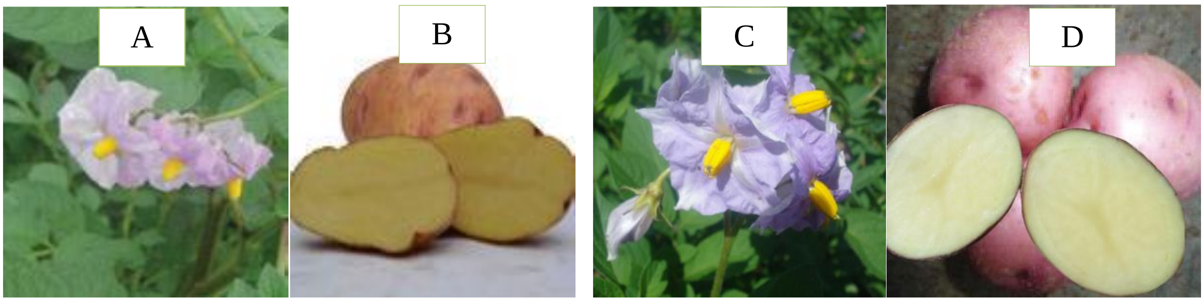


Plate 4.1: *Shangi* and *Kenya Karibu* varieties. A and B for *Shangi* flowers and tubers, C and D for *Kenya Karibu* flowers and tubers

4.2.3 *Ralstonia solanacearum* test in soil under field and semi-field experiments

Before experiments were set, soil test for *R. solanacearum* population density determination and identification were done by taking composite samples in all sites. The samples were analyzed at Biological science laboratory, Egerton University using serial dilution procedure as described by Kinyua *et al*. (2014). The population of *R. solanacearum* was calculated using the following formula (Fulya *et al*., 2020) :

$$\text{Colony forming unit (CFU/ml)} = \frac{Number\ of\ colonies\ counted \times dilution\ factor}{Quantity\ plated} \qquad 4.1$$

4.2.4 Field experimental procedure

After land preparation, canola seeds were sown on (3rd August 2019) at Mau-Narok site and (12th September 2019) at Elburgon at a rate of 6 kg ha^{-1} as recommended by Agriculture, Forestry and Fisheries, (2019) and allowed to grow for a period of two months. Experiment was laid out in randomized complete block design split-plot arrangement, canola green manure as main plot and combination of variety and fertilizer treatments as sub-plots replicated three times. Weed management was done in all plots (the one with green manure and the one without green manure). At early flowering stage (60 days after sowing), canola was uprooted, chopped (Approximately 1 cm) and incorporated into the soil in equal quantities (Mau-Narok 100 g m^{-2} and Elburgon 300g m^{-2}) (based on each site's productivity) at depth of 15 cm (McGuire, 2016). Canola production at Mau-Narok was low because of heavy rainfall that washed away seeds before germination. The whole plot (with and without green manure) was covered with a polyethylene sheet for two weeks to avoid glucosinolate compounds from volatilization (Sintayehu *et al*., 2014). Two weeks after incorporation, certified potato seeds *Shangi* and *Kenya Karibu* varieties sourced from Agricultural Development Cooperation (ADC) Molo were planted at a spacing of 75 cm × 30 cm (75 cm between the rows and 30 cm between the plants) with planting depth of 10 cm. The plot size was 3×1.5 m with 4 rows and 5 plants per row. Fertilizer treatments of (NPK+Ca+Mg+) **(**Table 4.1**)** were applied in splits, two thirds at planting and one third at flowering stage (Adrien, 2013). Recommended DAP and CAN were used as positive controls at rate of 500 kg ha^{-1} during planting and 300 kg ha^{-1} at flowering stage respectively, while in negative control plots, no fertilizer was applied. The rates for fertilizer treatments were calculated according to farmers practice and recommended nitrogen rate (90 kg ha^{-1}) as indicated by NPCK (2013) and Nyongesa *et al*. (2008).

Table 4.1: Fertilizer rates treatments with nutrients (N, P and K) applied

Fertilizer	Treatments	kg ha^{-1} N	kg ha^{-1} P	kg ha^{-1} K
Compound fertilizer (NPK	F1	0	0	0
10:26:10+ Ca, S, Mg, Cu, Zn, B,	F2	25.0	65.0	25.0
Mn, Mo).	F3	57.5	149.5	57.5
	F4	90.0	234.0	90.0
DAP 18% N and 46% P as Basal	F5	90.0	230.0	-
and CAN 27% N as top dressing		81.0	-	-

CAN: Calcium ammonium nitrate, DAP: Diammonium phosphate

4.2.5 Semi-field experimental procedure.

Semi-field experiment (pots) was laid out in completely randomized design in factorial arrangement with three replicates. Treatment combinations are shown in table 4.2. Canola seeds were grown at Egerton University farm for two months following the same management as for field experiments. The treatments were four levels of canola and five fertilizer treatments. Pot sizes of 20 cm diameter and 30 cm height were filled with 5 kg of top soil infested with bacterial wilt disease. A hundred and twenty pots were used in this experiment. At early flowering stage (60 days after sowing) canola was chopped and mixed into the soil in the pots at the rate of 0, 50, 75, 100 g kg^{-1} of soil and then covered with polyethylene sheet for two weeks before planting of potato seeds (Sintayehu *et al*., 2014). Certified seed potato (*Shangi* and *Kenya Karibu* variety) was planted one in each pot and fertilizer treatments applied at five rates as shown in the (Table 4.1**)**. (NPK+Ca+Mg+) fertilizer was applied in two splits, two third at planting stage and the remaining at flowering stage while DAP was applied at planting followed by CAN at flowering. The fertilizer treatments applied in the pots, were calculated based on the amount of soil per hectare 2,000,000 kg ha^{-1} f.s (farrow slice) and converted to the amount of soil used in the pot (5 kg).

Table 4.2: Treatment combinations (Canola green manure, fertilizer and potato varieties) for semi-field experiment.

Treatment	**Combination**	**Treatment**	**Combination**	**Treatment**	**Combination**	**Treatment**	**Combination**
T1	V1F1G1	T11	V1F3G3	T21	V2F1G1	T31	V2F3G3
T2	V1F1G2	T12	V1F3G4	T22	V2F1G2	T32	V2F3G4
T3	V1F1G3	T13	V1F4G1	T23	V2F1G3	T33	V2F4G1
T4	V1F1G4	T14	V1F4G2	T24	V2F1G4	T34	V2F4G2
T5	V1F2G1	T15	V1F4G3	T25	V2F2G1	T35	V2F4G3
T6	V1F2G2	T16	V1F4G4	T26	V2F2G2	T36	V2F4G4
T7	V1F2G3	T17	V1F5G1	T27	V2F2G3	T37	V2F5G1
T8	V1F2G4	T18	V1F5G2	T28	V2F2G4	T38	V2F5G2
T9	V1F3G1	T19	V1F5G3	T29	V2F3G1	T39	V2F5G3
T10	V1F3G2	T20	V1F5G4	T30	V2F3G2	T40	V2F5G4

Key: V1: *Shangi*, V2: *Kenya Karibu*, F5: DAP 500 + CAN 300 kg ha^{-1}, F4: NPK 900 kg ha^{-1}, F3: NPK 575 kg ha^{-1}, F2: NPK 250 kg ha^{-1}, G1: no green manure, G2: green manure 50 g $kg^{-1}$of soil, G5: green manure 75 g $kg^{-1}$of soil, G5: green mnaure100 g $kg^{-1}$of soil.

4.2.6 Experimental management

Experiments were kept weed free by weeding, first at two weeks and the second at six weeks after emergence. Late blight was controlled by alternating *Equation pro* (Famoxadone 225 g kg^{-1}+Cymoxanil 300 g kg^{-1}) sprayed at the rate of 10 g/20 L of water and *Ridomil gold* MZ 68 WG (Metalaxyl-M 40 g kg^{-1}+Mancozeb 640 g kg^{-1}) sprayed at a rate of 50 g/20 L of water. Spraying was done weekly during intensive rainy period and at two-week intervals in sunny period. Pests of canola such as aphids and flea beetles among others were monitored and controlled using Cypertox *250Ec (cyhalothrin* 25 g l^{-1}*)*. Canola pest attack were severe at early stage (2-4 weeks after planting), regular monitoring was done and sprayed twice depending on the attack.

4.2.7 Data collection

Data on *Ralstonia solanacearum* population density in the soil was collected at potato flowering stage and seven days after harvesting in both experiment (field and semi-field). Composite soil samples were collected from selected treatments, (DAP 500 kg ha^{-1}, NPK 900 kg ha^{-1}, 0 fertilizer) with green manure and without green manure application). The soil samples were analyzed using procedure of Kinyua *et al*. (2014). After two days of incubation, colony forming unit (CFU/ml) were counted and the actual (CFU) in original sample calculated using formula (4.1).

Under field experiment, disease incidence was monitored weekly by counting number of symptomatic plants in each plot based on a sample of 10 plants from middle rows. Disease severity was assessed 10 plants from middle rows using a scale of 0 - 4 disease, where 0 = no symptoms of wilting, 1 = 0 to 25% of stems showing wilting, 2 = 26 to 50% of stems showing wilting, 3 = 51 to 75% of stems showing wilting and 4 = 76 to 100% showing wilting (Yadessa, 2010). Disease incidence was calculated as a percent of diseased plants over the total number of plants. Disease severity grades were converted to percentage severity index (PSI) using the following formula (Sintayehu *et al.*, 2014; Yadessa, 2010):

$$PSI = \frac{\sum individual\ numerical\ rating}{Total\ number\ of\ plant\ assessed \times maximum\ score\ in\ the\ scale} \times 100 \qquad 4.2$$

4.2.8 Data analysis

Normality test was done for all data collected (under field and semi-field experiments), and the appropriate transformation (square root) was done to achieve normal distribution and meet the assumptions of ANOVA. General Linear Model (GLM) procedures of SAS (9.3) was used for ANOVA at Pr≤0.05 (Gomez and Gomez, 1984). The significantly different treatment means were separated using Tukey's honestly significant difference (HSD) test at 5% level of significance (Haynes, 3013).

Stastical Model (semi-field experiment):

$Y_{ijkl} = \mu + G_j + F_k + FG_{jk} + V_l + FV_{kl} + GS_{jl} + FGV_{jkl} + \varepsilon_{ijkl}$

Where:

Y_{ijkl} = Overall observations

μ = Overall mean

G_j= Effect due to j^{th} green manure levels

F_k= Effect due to $k^{\mathbf{th}}$ fertilizer levels

FG_{jk}= Interaction effect due to fertilizer and green manure

V_l= Effect due to l^{th} variety

FV_{kl}= Interaction effect due to fertilizer and variety

Statistical model (Field experiment)

$Y_{ijklm} = \mu + L_i + B_j + G_k + GL_{ik} + G\beta L_{ijk} + V_l + VL_{il} + VG_{lk} + VGL_{ikl} + F_m + FL_{im} + FG_{km} + FGL_{ikm} + FV_{lm} + FVL_{ilm} + FGV_{klm} + FGVL_{iklm} + \varepsilon_{ijklm}$

Where:

Y_{ijkl}= Overall observations

μ= Overall mean

L_i= Effect due to i^{th} location

B_j= Effect of j^{th} block

G_k = Effect due to kth green manure level

GL_{ik} = Effect due to green manure and locations interaction

$G\beta L_{ijk}$ = main plot error

V_l= Effect due to l^{th} varieties

VL_{il} = Interaction effect due to varieties and locations

VG_{kl}= Interaction effect due to varieties and green manure

VGL_{ikl} = Interaction effect due to varieties, green manure and locations

F_m= Effect due to m^{th} fertilizer rates

FL_{im}= Interaction effect due to fertilizer rates and locations

FG_{km}= Interaction effect due to fertilizer rates and green manure

FGL_{ikm}= Interaction effect due to fertilizer rates, green manure and locations

FV_{lm}= Interaction effect due to fertilizer rates and varieties interaction

FVL_{ilm}= Interaction effect due to fertilizer rates, varieties and locations

FGV_{klm}= Interaction effect due to fertilizer rates, green manure and varieties

$FGVL_{iklm}$= Interaction effect due to fertilizer rates, green manure, varieties and locations

ε_{ijklm}= Random error

4.3 Results

4.3.1 Effect of green manure and fertilizer treatments on *Ralstonia solanacearum* population density in the soil

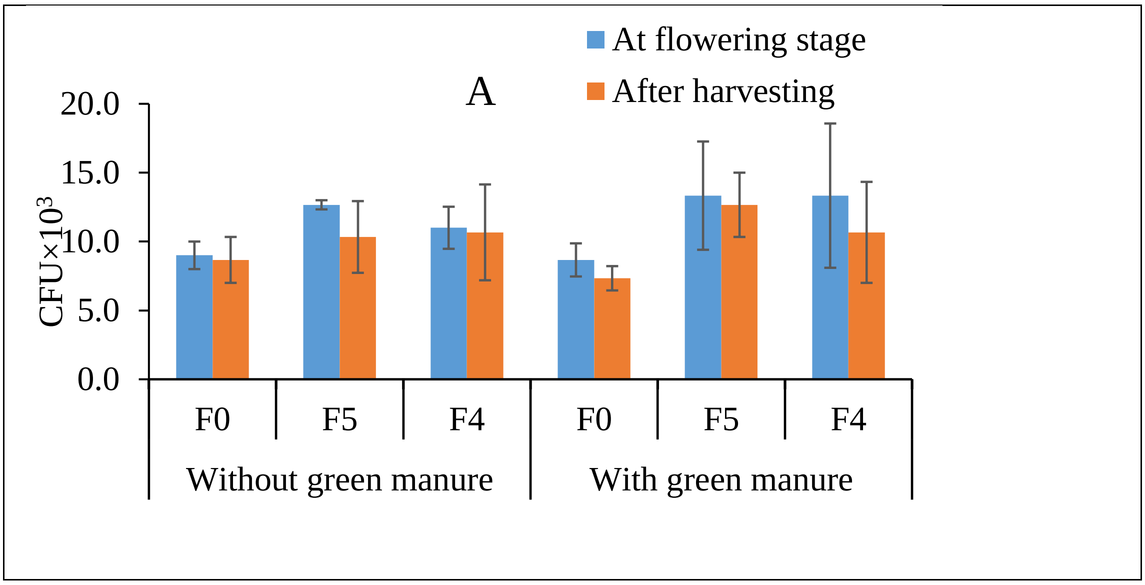
Preliminary soil sample analyses for bacterial population density were done in all sites and the results showed that they were 10.3×10^3, 11.5×10^3, and 7.5×10^3 colony-forming unit per milliliter (CFU/ml) at Mau-Narok, Egerton and Elburgon respectively. The soil at Mau-Narok site had high level of bacterial population density compared to the other sites.

The results showed that canola green manure and fertilizer did not have a significant effect on soil bacterial population density at $P<0.05$ under field and semi-field experiments. The higher soil bacterial population density was observed in two fertilizer treatments (NPK 900 kg ha^{-1} and DAP 500 kg ha^{-1}) compared to control and the trend was the same across the sites and semi-field experiment (Table 4.3and 4.4). The interaction between fertilizer levels and canola green manure did not have significant effect on bacteria population density at $P<0.05$ and interaction of NPK 900 kg ha^{-1}, DAP 500 kg ha^{-1} with canola green manure had the highest level of bacteria density while control had the lowest. The same trend was observed across all sites and semi-field experiment (Figure 4.1 and 4.2). The population density (CFU) increased as the fertilizer levels were varied 0-900 kg ha^{-1}, the highest increase was observed as in the following combinations; GM100 g kg^{-1} of soil with NPK 900 kg ha^{-1} followed by GM0 g kg^{-1} of soil with NPK900 kg ha^{-1} and GM 50 g kg^{-1} of soil with DAP 500 kg ha^{-1}. However, the lowest population was observed on green manure treatments combined with zero fertilizer application; GM 100 g kg^{-1} of soil with 0 fertilizer, followed by GM50 g kg^{-1} of soil with 0 fertilizer and GM75 g kg^{-1} of soil with 0 fertilizer (Figure 4.3). Unexpectedly, the high reduction of bacterial population density was observed between the samples taken before experimental setup and at flowering stage, with slight reduction on samples collected after potato harvesting 89%, 55.73% and 90.70 % at Mau-Narok, Elburgon and Egerton (semi-field experiment) respectively (Figure 4.4).

Table 4.3: Effect of canola green manure and fertilizer levels on *Ralstonia solanacearum* population density in the soil under field experiment

	Mau-Narok (CFU/ml×10^3)		Molo (CFU/ml×10^3)	
Fertilizer treatments	At flowering stage	After harvesting	At flowering stage	After harvesting
NPK 900 kg ha^{-1}	12.17^a	10.50^a	4.03^a	3.40^a
DAP500 +CAN300 kg ha^{-1}	13.00^a	11.50^a	3.70^a	3.07^a
Control	8.83^a	8.00^a	2.22^a	2.18^a
MSD	*8.91*	*7.90*	2.26	*2.61*
Canola green manure treatments				
Green manure	11.78^a	10.22^a	3.53^a	3.06^a
No green manure	10.89^a	10.89^a	3.10^a	2.71^a
MSD	*5.87*	*5.21*	*1.49*	*1.72*

The means followed by the same letter in same column are not significantly different at P <0.05, *DAP*: Diammonium phosphate, *MSD*: minimum significance difference, CFU: colony-forming unit.



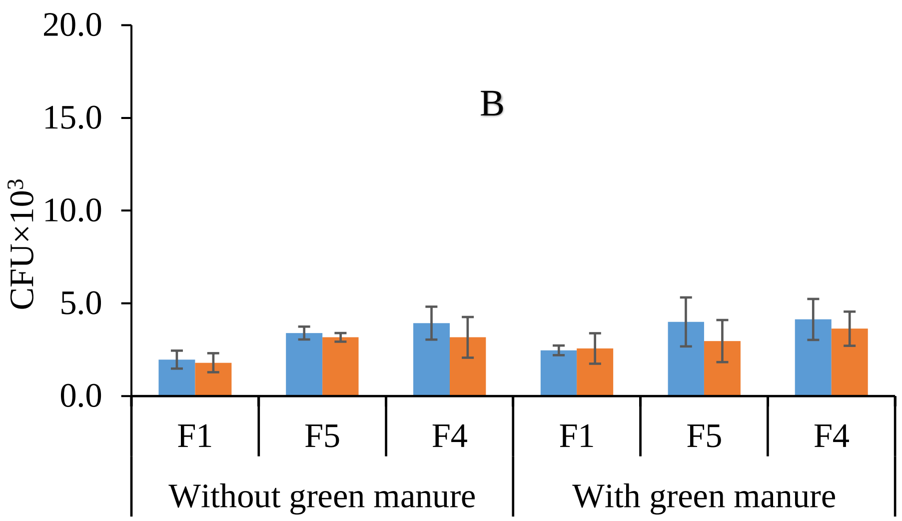


Figure 4.2: Effect of selected interactions between fertilizer and canola green manure treatments on *Ralstonia solanacearum* population density under field experiment. *A*: Mau-Narok, *B*: Elburgon at flowering stage. *CFU*: Colony forming Unit, F1: control, F5: DAP (Diammonium phosphate) 500 kg ha^{-1}, F4: NPK 900 kg ha^{-1}.

Table 4.4: Effect of canola green manure and NPK fertilizer on Ralstonia solanacearum population density under semi-field experiment (CFU/ml×10^{3}).

	At flowering stage	After harvesting
Fertilizer treatments		
NPK 900 kg ha^{-1}	1.22^{a}	0.98^{a}
DAP 500 +CAN300 kg ha^{-1}	1.20^{a}	0.97^{a}
Control	0.79^{a}	0.65^{a}
MSD	***0.43***	***0.38***
Canola green manure treatments		
GM 100 g kg^{-1} of soil	1.09^{a}	0.85^{a}
GM 75 g kg^{-1}of soil	1.06^{a}	0.86^{a}
GM 50 g kg^{-1}of soil	1.08^{a}	0.86^{a}
GM 0 g kg^{-1}of soil	1.04^{a}	0.89^{a}
MSD	***0.55***	***0.49***

The means followed by the same letter in the same column are not significantly different at P <0.05. *GM*: canola green manure, *DAP*: Diammonium phosphate, *MSD*: minimum significance difference, CFU: colony-forming unit.

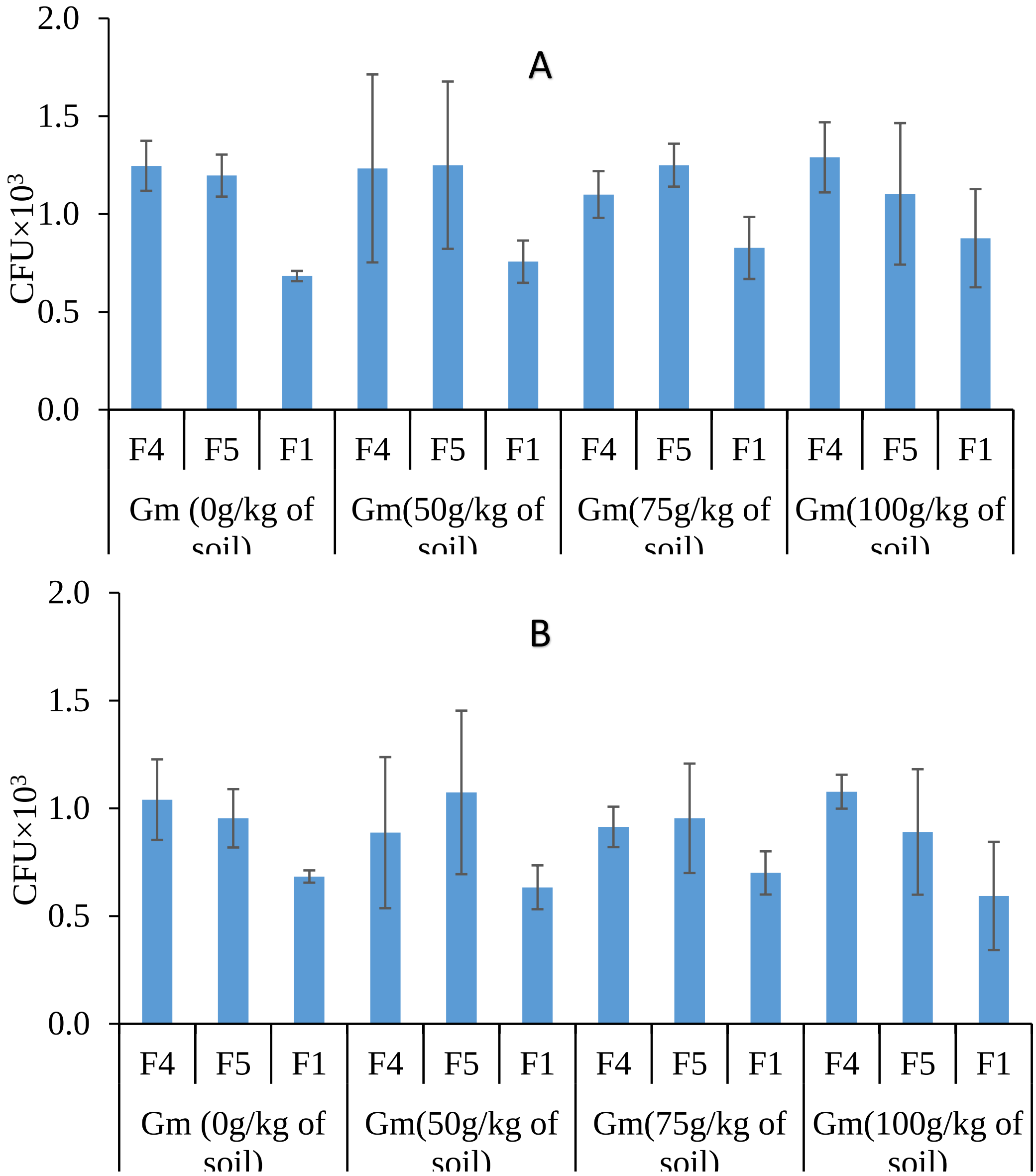


Figure 4.3: Effect of selected interaction between fertilizer and canola green manure on *Ralstonia solanacearum* population density under semi-field experiment. (*A)*: soil sample collected at potato flowering stage, *(B)*: soil sample collected after potato harvesting, *CFU*: Colony forming Unit, Gm: canola green manure in grams per kilogram of soil. F1: control, F5: DAP (Diammonium phosphate) 500kg ha^{-1}, F4: NPK 900 kg ha^{-1}.

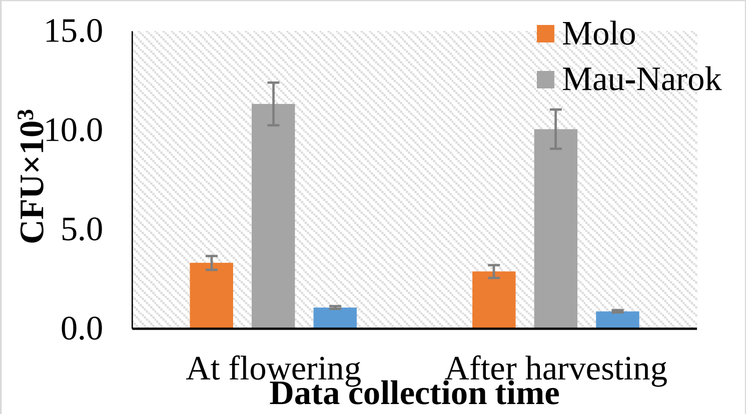


Figure 4.4: Means and standard error bars of *Ralstonia solanacearum* population density as affected by fertilizer and canola green manure treatments at different sites at different sampling times during potato growth (combined data for fertilizer and canola green manure).

4.3.2 Effect of canola green manure and fertilizer treatments on disease incidence and severity

The result shows that fertilizer and canola green manure had no significant (at 5% level of significance) effect on disease severity and disease incidence in all sites. Even though no significant difference was observed statistically, NPK 900 kg ha^{-1} and DAP500 kg ha^{-1} treatments had higher level of potato bacterial wilt incidence and severity than control. High level of disease severity and incidence were observed on the plant from the treatments NPK 900 kg ha^{-1} followed by DAP 500 kg ha^{-1} at Mau-Narok site with slight difference at Elburgon, because NPK 900 kg ha^{-1} was followed by control (Table 4.5 and 4.6). Interaction between fertilizer treatments and green manure levels, seems to be influenced by fertilizer levels, because the two treatments NPK 900 kg ha^{-1} and DAP500 kg ha^{-1} exhibited the high level of potato plant incidence interacted either with or without canola green than no fertilizer application (Figure 4.4). Mau-Narok site exhibited high level of potato plant incidence and severity than Elburgon site but this was due to differences observed initially before experimental set up. The disease incidence seems to be positively correlated to disease severity in both sites. Potato varieties showed differences, *Shangi* was most susceptible variety while *Kenya Karibu* did not show any symptom of wilting and the same trend was observed across the sites.

Table 4.5: Effect of canola green manure and fertilizer treatments on potato bacterial wilt disease incidence (Shangi variety)

Fertilizer treatments	Mau-Narok (%)	Elburgon (%)
NPK 900kg ha^{-1}	28.61[a]	4.26[a]
DAP500 +CAN300kg ha^{-1}	27.41[a]	0.83[a]
NPK 575 kg ha^{-1}	19.44[a]	0.83[a]
NPK 250 kg ha^{-1}	18.58[a]	1.76[a]
Control	15.65[a]	2.50[a]
MSD	*17.65*	*5.59*
Canola green manure treatments		
Green manure	23.05[a]	2.74[a]
No green manure	20.83[a]	1.33[a]
MSD	*24.82*	*10.09*

The means followed by the same letter in the same column are not significantly different at P <0.05. *DAP*: Diammonium phosphate, *CAN*: Calcium ammonium nitrate, *MSD*: minimum significance difference.

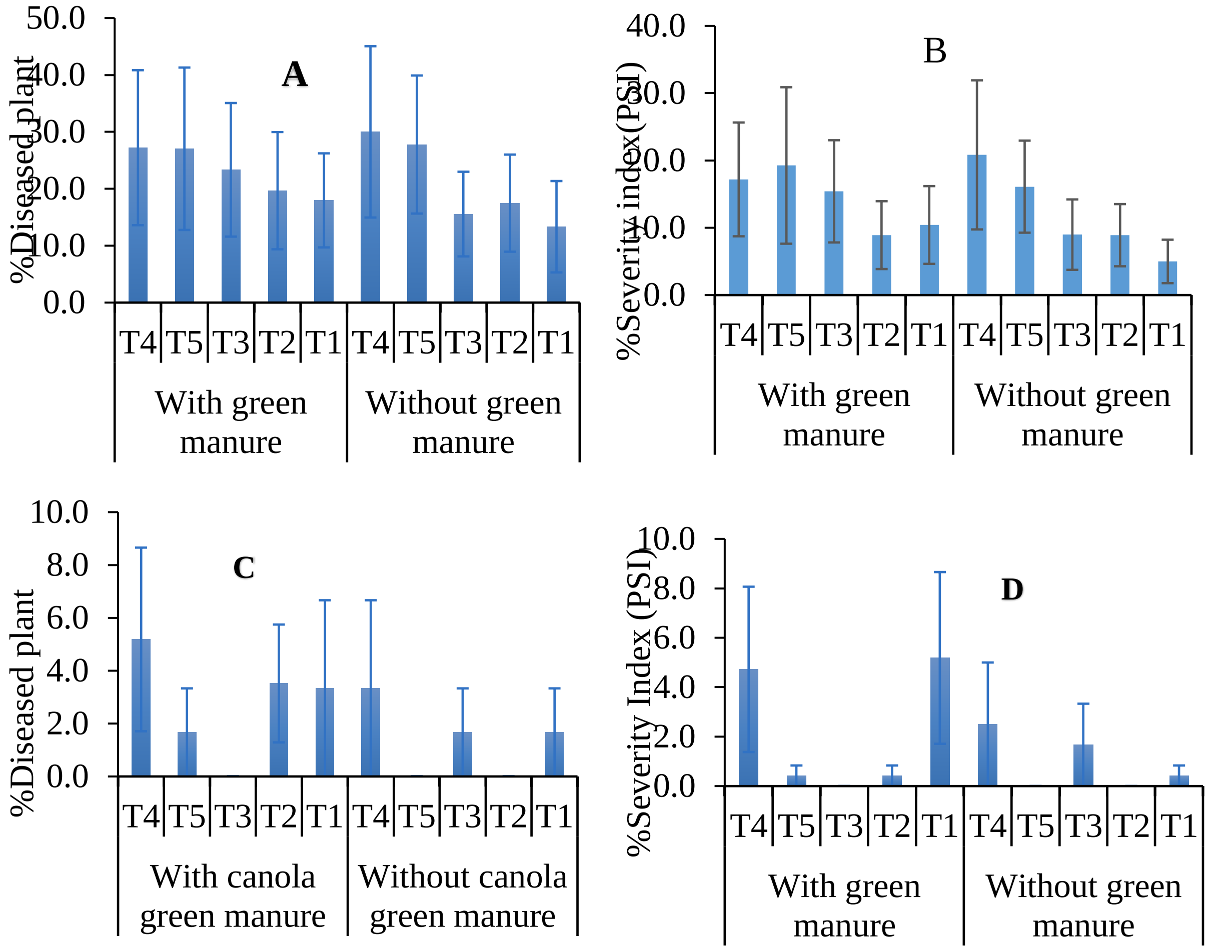


Figure 4.5: Effect of interaction between fertilizer and canola green manure on potato bacterial wilt incidence and severity (Data for *Shangi* variety). A and B are for Mau-Narok site, C and D are for Elburgon site. F1: control, F5: DAP (Diammonium phosphate) 500 kg ha^{-1}, F4: NPK 900 kg ha^{-1}.

Table 4.6: Effect of canola green manure and fertilizer treatments on potato bacterial wilt disease severity (Data for Shangi variety)

Fertilizer treatments	Mau-Narok (PSI)	Elburgon (PSI)
NPK 900kg ha^{-1}	19.01^a	3.61^a
DAP500 +CAN300 kg ha^{-1}	17.69^a	0.21^a
NPK 575 kg ha^{-1}	12.20^a	0.83^a
NPK 250 kg ha^{-1}	8.91^a	0.21^a
Control	7.71^a	2.80^a
MSD	*13.24*	*4.37*
Canola green manure treatments		
Green manure	14.24^a	2.15^a
No green manure	11.97^a	0.92^a
MSD	*19.01*	*8.52*

The means followed by the same letter in the same column are not significantly different at P <0.05. *PSI:* Percentage severity index, *DAP*: Diammonium phosphate, *CAN*: Calcium ammonium nitrate, *MSD*: minimum significance difference**.**

4.4 Discussion

4.4.1 Effect of canola green manure and fertilizer treatments on *Ralstonia solanacearum* population density in the soil, bacterial wilt incidence and severity

The results show that, canola green manure did not have a significant effect on *Ralstonia solanacearum* population density in the soil, bacterial wilt incidence and severity. The observed increase in bacterial density in the soil were mostly associated with plant growth and vigour due to nutrients. Zheng *et al.* (2019) found that nitrogen content was positively correlated (r^2=0.2659) with *Ralstonia solanacearum* abundance, this could be the reason why NPK 900 kg ha^{-1} and DAP500 kg ha^{-1} treatments had higher bacterial population, incidence and severity compared to control.

The occurrence and prevalence of soil-borne diseases including bacterial wilt are closely related to soil quality (Shen, 2018). Messiha *et al.* (2007) on the study that was evaluating the effect of soil amendments and management on potato brown rot (*Bacterial wilt*) incidence and severity, reported that NPK fertilizer application may reduce or increase disease depend on some soil condition. They found that, NPK treatment amended in sandy soils significantly increased the percentage-wilted plants from 8.3% to 28.4% and wilt severity from 4.3% to 27.1% in clay soils. They also found higher *Ralstonia solanacearum* population, disease severity and incidence in organically amended soil, and then concluded that it may be due to the high availability of substrate. Although, there is no clear literature focusing on relationship between soil fertility, potato growth and bacterial wilt incidence and severity.

The findings on the biofumigation potential in management of disease has been inconsistent. Njoroge *et al*. (2008) found that canola residue had higher population of *Pseudomonas* spp compared to other treatments and no canola application. However, there are some other studies revealed that canola suppressed growth of soil pathogen mostly for fungus. Motisi *et al*. (2009), evaluated the effect of different parts of *Brassica juncea* green manure, above-ground parts (AP), below-ground parts (BP) and combination of both (AP+BP). He found that AP and AP+BP suppressed *Rhizoctonia solani* by 54 and 63%, respectively, and *Gaeumannomyces graminis* var. *tritici* by 40 and 40%, respectively, compared with control. Fulya *et al.* (2020) found that green manure of yellow mustard (*Sinapis alba*), turnip (*Brassica rapa*), arugula (*Eruca vesicaria*), Mighty mustard (*B. juncea*), rape (*B. napus*,), mustard green (*B. carinata*) and brown mustard (*B. juncea*) cover crops were effective in suppressing *Rhizoctonia solani* and *Phytophthora nicotianae.* Larkin and Griffin (2007) evaluated the effect of brassica crops, including canola, rapeseed, radish, turnip, yellow mustard, and Indian mustard on potato soil borne pathogens. Found that the growth of (*Rhizoctonia solani*, *Phytophthora erythroseptica*, *Pythium ultimum*, *Sclerotinia sclerotiorum*, and *Fusarium sambucinam)* inhibited by barassica crops, with the highest inhibition of (80% to 100%) resulted from Indian mustard application under *in-vitro* experiment. In the same study, under field experiment, Indian mustard was most effective for reducing powdery scab and common scab diseases, whereas rapeseed and canola were most effective in reducing *Rhizoctonia* diseases. In this study, observed inefficacy of canola green manure may be associated with low glucosinolate contents compared to other brassica family. Kirkegard (1998) reported that biofumigation potential varies from species to species, he found that oriental or Indian mustard (*Brassica juncea*) has the highest biofumigation potential than rapeseed and canola (*Brassica napus*). This was supported by Larkin *et al*. (2018) who reported that canola has low, rapeseed moderate and mustard high level of glucosinolates. Furthermore, canola has been bred from rapeseed first around 1960s (Oplinger *et al*., 1989) for reducing erucic acid content and glucosinolate contents in the oil and feedstuffs. These compounds are believed to cause diseases to animals when in higher quantity. Canola has less than 30 micromoles of glucosinolates and less than 2% of erucic acid reported Canola Council of Canada (2020) and this was confirmed by Oplinger *et al*. (1989) who reported that contains less than 2% erucic acid and the meal less than 3 mg/g of glucosinolates.

Overall observation of bacterial population density reduction was observed across sites. The high reduction was observed on the samples taken at potato flowering stage compared to initial samples before experiment set up. This reduction may be as a result of solarization produced by a black polyethylene sheet used to cover experiment after incorporation of canola green manure for a period of two weeks as recommended by Fulya *et al*. (2020) and Sintayehu *et al*. (2014) to void volatilization of glucosinolate compound. Soil solarization is based on the use of solar energy to raise soil temperature by 10 to 15°C to the normal and this may affect the survival of the soil pathogen even at depths of approximately 20 cm (Kanaan *et al*., 2015). *Ralstonia solanacearum* is very sensitive to soil temperature fluctuation, the temperature below 20℃ and above 35℃ affect its growth (Dinesh *et al*., 2014). Jirasak and Jirasak (2007) on the study that was evaluating effect of heat treatment on *Ralstonia* spp, found that the bacteria populations were reduced from $2.4\text{-}7\times10^{8}$ colony forming units (CFU) g^{-1} to 0-115, and 11-173 CFU g^{-1} under 60℃ for 120 min and 45℃ for 48 hours respectively and reduced bacterial wilt incidence by 50- 75%. Patrício *et al*. (2005) on the study that was evaluating the effect of soil solarization for the control of *R. Solanacearum,* found that no wilted tomato plants in the plots solarized for 60 days. The same result was observed by Vinh *et al.* (2005) who found that soil solarization for 60 days using transparent plastic mulches reduced the incidence of bacterial wilt of tomato.

Two potato varieties *Kenya Karibu* and *Shangi* were used in this study and *Kenya Karibu* showed to be tolerant to bacterial wilt disease than *Shangi*. *Kenya Karibu* did not show any symptom of bacterial wilt in all sites and semi-field experiments. It is reported that there is no potato variety that has been found to be resistant to bacterial wilt 100%, but some level of tolerance may be observed (Felix *et al*., 2010; NPCK, 2019). The absence of potato varieties resistance to bacterial wilt could be attributed to the high genetic variability of strains within the *R. solanacearum* species complex (Hartman & Elphinstone, 1994). Through breeding, moderate to highly resistant potato varieties have been released, though those varieties are not effective against all the strains of the pathogen and high frequency of latent infection in tubers is still a major problem in breeding program (Patil *et al*., 2012). Felix *et al*. (2010) on the study that was assessing tolerance of potato cultivars (*Tigoni, Asante, Kenya Karibu, Kenya Sifa* and *Dutch Robjyn*) on bacterial wilt in Kenya, found that cultivars like *Kenya Sifa* and *Kenya Karibu* were the most tolerant to bacterial wilt, while

Dutch Robjyn and *Tigoni* were the most susceptible to bacterial wilt disease. This was confirmed by Muthoni *et al*. (2014), who found that *Kenya Karibu, Kenya Sifa* and *Ingabire* were three most bacterial wilt resistant potato genotype among thirty-six evaluated potato genotypes. Even if no physical symptoms, tolerant varieties carry latent infection to the next progeny once planted as seeds (Priou *et al.,* 1996). French (1996) reported the same that potato varieties were found to have some degree of tolerance to potato bacterial wilt but it not safe because it may carry latent infections to the next progeny.

4.5 Conclusion

In the present study, canola green manure and compound NPK fertilizer application in two agro-ecological zones in Nakuru, did not show a promising result in management of potato bacterial wilt. This may be associated with low content of active compounds in canola (glucosinolate compounds). *Kenya Karibu* variety showed a potential to be tolerant to bacterial wilt disease than *Shangi* and this may be exploited by breeders to produce a resistant cultivar. A significant reduction of bacterial population density in the soil at potato flowering stage was observed 89.00, 55.73 and 90.70% at Mau-Narok, Elburgon and Egerton sites respectively and this might be associated with the effect of polyethylene cover used for two weeks. The effect of polyethylene cover on bacterial wilt pathogen reduction in the soil may be evaluated further over time.

CHAPTER FIVE

EFFECT OF COMPOUND FERTILIZER AND CANOLA GREEN MANURE ON GROWTH, NUTRIENT USE EFFICIENCY AND POTATO (*Solanum tuberosum*.L) TUBER YIELD

Abstract

Continuous cultivation using nitrogen-phosphorus (Diammonium phosphate: DAP) only fertilizer may be led to depletion of other macro and micronutrients in potato production areas of Kenya. Purpose of the study was to assess alternative soil amendments including use of multi-nutrient compound fertilizer and canola green manure in potato production. Two sites field and semi-field experiments were conducted in central Rift Valley during the August-December 2019 season using two potato varieties (*Shangi* and *Kenya Karibu*). Canola green manure levels, four levels of fertilizer (NPK+Ca+Mg+) applied at a rate of 0 kg ha^{-1} (F1), 250 kg ha^{-1} (F2), 575 kg ha^{-1} (F3), 900 kg ha^{-1} (F4) and diammonium phosphate (DAP 500 kg ha^{-1}) +Calcium ammonium nitrate (CAN300 kg ha^{-1}) (F5) as a positive control were used. Field experiment was carried out in randomized complete block design, whereas pot was conducted in completely randomized design. The results indicated that fertilizer F4 increased potato tuber dry weight and plant height by 5.0% in both cases respectively over the normal recommended F5 under field experiments. Treatment F4 also increased nutrients (nitrogen, phosphorus and potassium) uptake by 13%, 26% and 3% respectively under field experiment as compared to F5. Generally, canola green manure did not show a significant effect on potato plant height and yield, though F4 with green manure exhibited an increase of 7% and 38% on plant height and tuber dry weight respectively. The study recommends the use of F4 for potato production in Kenya and further recommends additional research to assess compound fertilizers over more seasons with monitoring and evaluation of their effect on soil physical and chemical properties.

5.1 Introduction

Potato (*Solanum tuberosum*.L) is a staple food in Kenya, and approximately 800,000 farmers are involved in the production generating about 500 million USD annually (AGRA, 2019). Kenya is among the top potato producing countries in Eastern Africa community with large production area, though potato production per hectare is declining year by year (FAOSTAT, 2020). Despite its importance, the production is far below the potential production. Diseases and poor soil fertility management are the major cause of potato yield reduction in Kenya. Bacterial wilt is the most prevalent disease, affecting 77% of potato farmers, followed by late blight (67%) and viral diseases (12%) (Muthoni *et al.*, 2013). Low soil fertility is another production constraint mainly due to continuous cropping without replenishing all mined nutrients. Potato production in Kenya is mostly occupied by smallholder farmers, with a land size below 2.02 hectares (Okello *et al*., 2016) leading to intensively land use without fallow. Major potato growing areas in Kenya has low soil phosphorus as 2.9 $mgkg^{-1}$ while total nitrogen is lower than 1.5 g kg^{-1}. In addition, the available fertilizer recommendations do not consider for potassium (K) yet some studies have indicated the benefit of potassium addition in potato production (Muthoni & Nyamongo**,** 2013).

In Kenya, potato farmers have been relaying on one source of nutrient Diammonium phosphate (DAP). Ammonium based fertilizer such as (DAP) dominate in the production of potatoes, and research has revealed that it has side effect of gradual soil acidification if continuously used over a long time (Maryanne *et al*., 2015; Muthoni, 2016). Despite acidification issues, DAP does not supply all essential nutrients that are required by potato in large quantities. Potato requires nitrogen (N), phosphorus (P), potassium (K), magnesium (Mg) and calcium (Ca) amongst other nutrients (Haifa, 2020).

Currently there are different types of NPK fertilizers with additional nutrients including micro- nutrients available in Kenya. However, a lack of information on the benefits of new fertilizer formulations, has limited their utilization. Other factors limiting the use of such fertilizers include access and availability. Apart from nitrogen (N), phosphorus (P), and potassium (K) as major nutrients, potato require other secondary macronutrients such as magnesium (40-60 kg ha^{-1}) and calcium (100-120 kg ha^{-1}) as reported by Haifa (2020). Calcium is a component of cell wall, it helps plants to resist stress condition and plant pathogens attack whereas magnesium, has a crucial role in photosynthesis, and is involved in production sugars and proteins. Annual magnesium application on potato increased the yield by 1- 10% (YARA, 2020). Recent studies focused on the importance of using fertilizes that contains only N, P and K nutrients in potato production. Adrien (2013) evaluated the effect of time and method of NPK 17-17-17 application, Adhikari (2009) evaluated the effect of effect of NPK (0:0:0, 50:50:50, 100:50:50, 100:75:50, 100:75:100, 100:100:100 and 150:100:100 N, P_2O_5 and k_2O kg ha^{-1}) on vegetative growth and yield. The present study was carried out to evaluate the effect of NPK 10:26:10 + (Ca, S, Mg, Cu, Zn, B, Mn, and Mo) fertilizer and canola green manure on growth, nutrient use efficiency and potato tuber yield.

5.2 Materials and Methods

5.2.1 Experimental site description (see section 4.2.1)

5.2.2 Germplasm description (see section 4.2.2)

5.2.3 Soil physical and chemical properties for the experimental sites

Before the experiments were set up, soils and canola green manure samples were sent to Kenya National Agricultural Research Laboratory (NARL) KALRO Kabete for physio-chemical analyses. Soil available nitrogen, available phosphorus, available potassium, calcium, magnesium, organic carbon, pH and soil texture were analyzed. Soil pH was determined using 1:2.5 ratio of soil: water by electrometric method, soil texture using Hydrometer method (Okalebo, 2002). Total nitrogen (TN) by wet-oxidation procedure of the Kjeldahl method (Sahlemedhin & Taye, 2000). Available (P, K, Ca and Mg) were analyzed using Mehlich double acid method (Mehlich *et al*., 1962) and total organic carbon using calorimetric method (acidified dichromate as an oxidizing agent) (Anderson *et al*., 1996). In all sites, the soils were found to be *Sand clay loam texture*. Based on potato nutrients requirement, phosphorus was low in all experimental sites and calcium was low at Elburgon sites. Soil pH was ideal for potato growth at Mau-Narok and Egerton sites (Table 5.1).

In addition, canola biomass (stem, leaves and flowers) samples were taken randomly from ten plants in the field. A sub sample of 500 g fresh biomass was sent to Kenya National Agricultural Research Laboratory (NARL) KALRO Kabete for total nitrogen, phosphorus, and potassium contents. The following principle was used during analysis; the samples were dried in oven at 70 ^{0}C and oxidized by hydrogen peroxide (30% H_2O_2) at relatively low temperature (100 ^{0}C). After decomposition of the excess H_2O_2 and evaporation of water, the digestion was completed by concentrated sulphuric acid at elevated (330 ^{0}C) temperature under the influence of Se (selenium) as a catalyst. Then the elements were determined using the following methods; potassium (K) was determined with a flame photometer, phosphorus (P) determined calorimetrically on spectrophotometer at 880nm wavelength, N-total measured by distillation followed by titration with 0.3N HCl (Walinga *et al*., 1995). The analysis indicated canola biomass contained 3.03% nitrogen, 0.45% phosphorus and 3.92% potassium.

Table 5.1: Soil physical and chemical properties of the experimental sites

Soil fertility factors	Mau-Narok	Egerton	Elburgon
Soil PH	5.68	5.47	4.57
Total nitrogen (gkg^{-1})	3.00	2.00	3.00
Total organic Carbon (gkg^{-1})	33.20	24.40	35.40
Available P ($mgkg^{-1}$)	5.00	15.00	5.00
Available K ($cmolkg^{-1}$)	1.48	1.34	0.94
Ca ($cmolkg^{-1}$)	6.20	4.60	0.60
Mg ($cmolkg^{-1}$)	2.24	2.03	1.36

P: phosphorus, ***Ca:*** Calcium, ***Mg:*** Magnesium.

5.2.4 Experimental procedures and management (See section 4.2.4, 4.2.5 and 4.2.6)

5.2.5 Data collection

a. Growth and yield parameters

Number of stems per plant was counted at 28, 35 and 42 days after emergence (DAE) and plant height was measured at 28, 35, 42 and 56 (DAE) using a ruler. Ten plants from the middle rows were uprooted per plot, number of tubers counted and average number tubers per plant calculated. Tuber grading was done by ranking tubers in three classes; big size: >60 mm diameter, medium size: 30-60 mm diameter- small size: <30 mm diameter (International Fertilizer Development Center, 2017). Different tuber grades were counted separately and converted into percentage out of total. Fresh tuber weight was taken after grading and sub samples of 500 g of fresh tuber were taken in each plot. The samples were dried in the oven at 70°C and then converted into tons per hectare.

b. Assessment of nitrogen, phosphorus and potassium uptake and use efficiency

At maturity stage, three plants from selected treatments (F4: NPK 900 kg ha^{-1}, F5: DAP 500+CAN 300 kg ha^{-1} and F1: control, with and without canola green manure) were uprooted for nitrogen (N), phosphorus (P), potassium (K) nutrients uptake analyses. Potato tuber samples of 50 g were dried in oven at 70 °C for 72 h. The samples were labeled and taken to NARL-KARLO Nairobi for N, P and K analyses using the methods described in the section 2.3. The nutrients uptake was calculated using the following formula:

$$\boldsymbol{Nutrients\ uptake\ (kgha^{-1})} = nutrients\% \times d.w\ (\ tubers) \qquad (5.1)$$

Where: D.w= Dry weight

Agronomic efficiency (or fertilizer use efficiency) was calculated using potato production excluding control, over total input applied (Badr *et al*., 2012):

$$\textbf{Agronomic efficiency}\ \frac{Yt-Y0}{F} \tag{5.2}$$

Where; Y_t = total tuber yield under treatment (kg ha^{-1}), Y_0 = total tuber yield under control (kg ha^{-1}), F = fertilizer applied (kg ha^{-1}).

Nutrient recovery was calculated using the following formula:

$$\textbf{Nutrient recovery} = \frac{Nt-N0}{N} \times 100 \tag{5.3}$$

Where: Nt = total nutrient uptake by crop under treatment (kg ha^{-1}), No = total nutrient uptake under control (kg ha^{-1}), N = Applied nutrient (kg ha^{-1}) (Badr *et al*., 2012).

5.2.6 Data Analysis

The data were subjected to normality test and the appropriate transformation (log or square root) was done to achieve normal distribution and meet the assumptions of ANOVA. Analysis of variance using (ANOVA) General Linear Model (GLM) procedures of SAS (9.3) at $P \leq 0.05$ was done (Gomez and Gomez, 1984). The significantly different treatment means were separated using Tukey's honestly significant difference (HSD) test at 95% level of confidence (Haynes, 2013). Pearson correlation analysis at 5% level of significance (Wang, 2013) was done to determine the relationship between tuber dry weight and other agronomic parameters (plant height, number of stems and number of tubers per plant). Statistical model used are the same for objective two, section 4.2.8.

5.3 Results

5.3.1 Potato growth response to fertilizer and Canola green manure application

a. Number of stems

The number of stems were not significantly affected by fertilizer treatments and canola green manure for field (Appendix 1) and semi-field experiments (Appendix 2). However, significant difference was observed between varieties; *Shangi* had higher number of stems (2-6) than *Kenya Karibu* (2-4) across sites and semi-field experiment.

b. Plant height

Plant height was significantly affected by fertilizer treatments at ($p \leq 0.001$) (Appendix1), where the trend was F4 (NPK: 900 kg ha-1) >F5 (DAP: 500 kg ha-1)> F3 (NPK: 575 kg ha-1) and the lowest was from F2 (NPK: 250 kg ha-1) and control (F1). F4 treatment increased plant height by 4.0% and 46.0% compared to positive control (F5) and negative control (F1) respectively. Plant height differed by varieties; Shangi (22-81 cm) compared to Kenya Karibu (17-63 cm) under field conditions. Canola green manure treatments did not show a significant effect on plant height, though green manure increased plant height by 4.4% compared to none green manure under field condition. Plants treated with F4 fertilizer treatment had the highest plant heights of 63 cm, 59 cm and 41 cm at Mau-Narok, Elburgon and semi-field experiment respectively while untreated plots had 48 cm, 35cm and 23cm respectively.

The sites exhibited different plant heights at 28, 42 and 56 days after emergence (DAE) under field condition. Plants at Mau- Narok exhibited higher plant height than Elburgon site. At Mau-Narok site, for *Shangi* variety, plant height was significantly affected by fertilizer levels for all days i.e; 28, 35, 42 and 56 Days after emergence (DAE) (Appendix 3). The highest plant heights of 75 cm, 72 cm and 71 cm were recorded for treatments treated with F4, F5 and F3 respectively and control had the lowest plant height (Figure 5.1A). For *Kenya Karibu*, fertilizer treatment had significant effect on plant height at 35, 42, and 56 DAE. *Kenya Karibu* responded late to fertilizer due to its slow growth and root establishment. The highest plant heights of 50 cm, 47 cm, and 45 cm were recorded for treatments with F4, F3, and F5 respectively for *Shangi* and control had the lowest value (Figure 5.1B). Canola green manure had no significant difference on plant height for all varieties, though the highest values were recorded where green manure was applied.

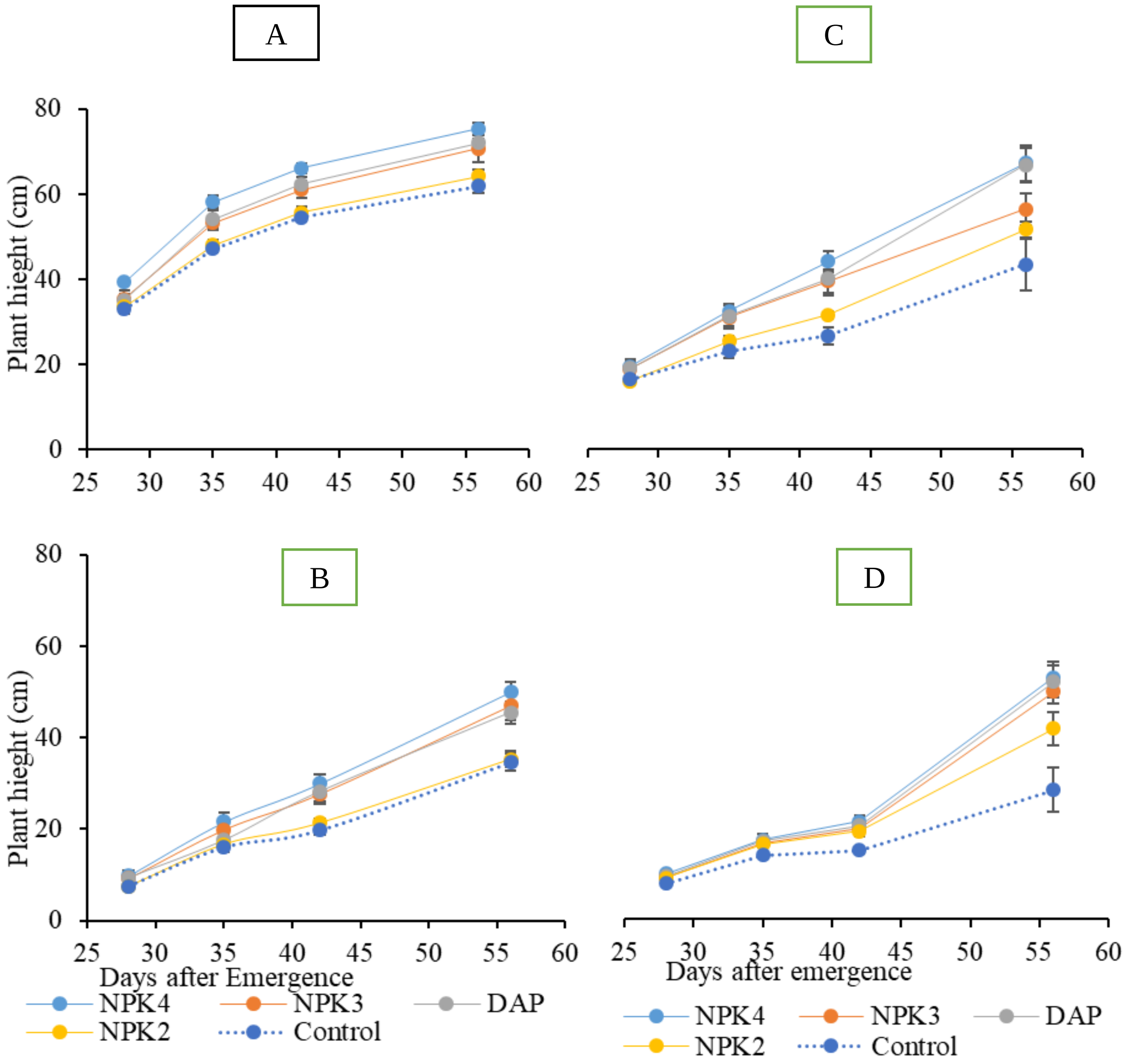


Figure 5.1: Fertilizer treatments effect on potato plant height (cm) for field experiment. A (*Shangi*) and B (*Kenya Karibu*) are for Mau-Narok site, C (*Shangi*) and D (*Kenya Karibu*) are for Elburgon site. NPK4: NPK 900 kg ha^{-1} (F4), NPK3: NPK 575 kg ha^{-1} (F3), NPK2: NPK 250 kg ha^{-1} (F2), DAP: DAP 500 kg ha^{-1} + CAN 300 kg ha^{-1} (F5), Control (F1)

At Elburgon site, plant height was significantly affected by fertilizer for data collected at 35, 42 and 56 (DAE) (Appendix 4), while canola green manure did not have significant effect on plant height. The highest plant heights (66 cm, 65 cm and 55 cm for *Shangi* variety; 55 cm, 52 cm and 49 cm for *Kenya Karibu* variety) were recorded for treatments treated with F4, F5 and F3 respectively. The control had the lowest values (Figure 5.1C and D). The same trend was observed in semi-field experiment; fertilizer treatments had a significant effect on potato plant height, means separations shows that, F4, F3 and F5 treatments had the highest plant height 47 cm, 44 cm and 41 cm respectively at 56 (DAE). Variety differences were observed as for field experiments, *Shangi* had the highest plant height over *Kenya Karibu* (Figure 5.2). Canola green manure levels did not show any effect on plant height.

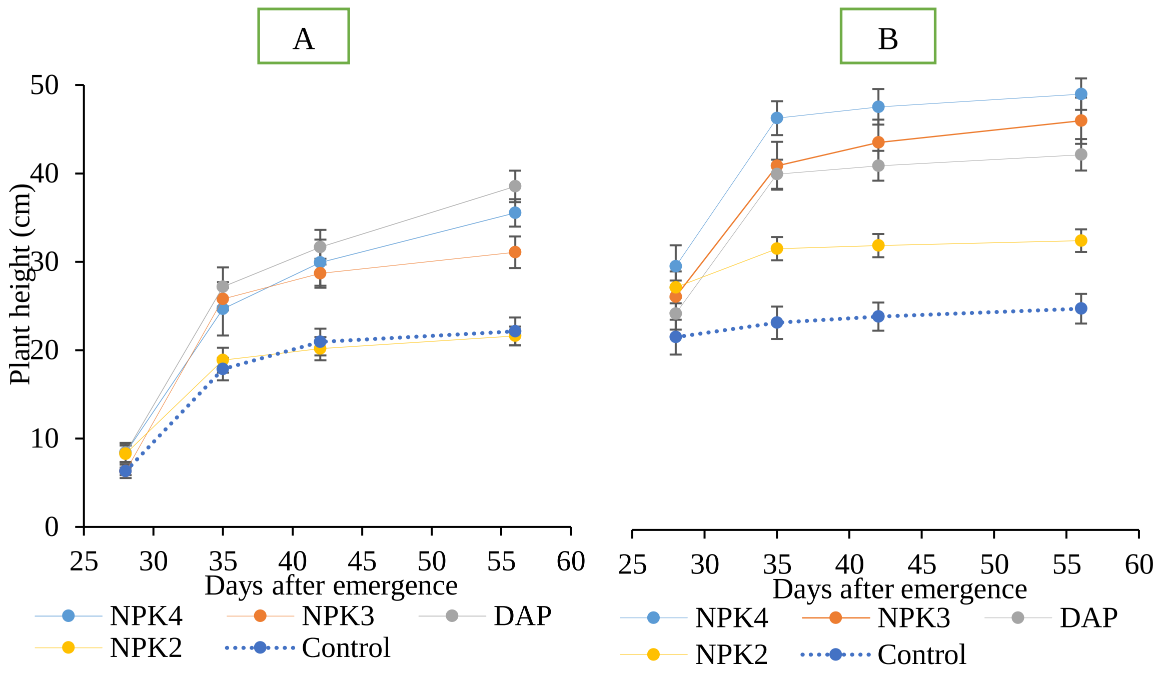


Figure 5.2: Fertilizer effect on potato plant height (cm) for semi-field experiment. (A: *Kenya Karibu*, B: *Shangi*). NPK4: NPK 900 kg ha^{-1} (F4), NPK3: NPK 575 kg ha^{-1} (F3), NPK2: NPK 250 kg ha^{-1} (F2), DAP: DAP 500 kg ha^{-1} + CAN 300 kg ha^{-1} (F5), Control (F1)

5.3.2 Effect of canola green manure and fertilizer treatments on potato tuber yield; number of tubers and tuber dry weight

Number of tubers and tuber dry weight were significantly affected by fertilizers treatments under field (p=0.001) and semi-field experiments (p=0.001) (Appendices 5 and 2). Canola green manure did not show significant effect on number of tubers and tuber dry weight as well, though an increase of 11% in tuber dry weight and 5% in number of tubers due to green manure application were observed. The highest number of tubers; 10, 9 and 9 per plant were from F5 followed by F4 and F3 treatments respectively, whereas the highest dry tuber weight; 8.19 t ha^{-1}, 7.82 t ha^{-1}, 6.41 t ha^{-1} were from F4 followed by F5 and F3 treatments respectively. There was an increase of 5% and 83% of tuber dry weight from F4 treatment over positive control F5 and negative control F1 respectively. Varietal differences were observed on number of tubers but not significant on dry tuber weight, *Shangi* had high number of tuber (9.19) than *Kenya Karibu* (7.53). In each site, variety and fertilizer treatment had a significant effect on number tubers and tuber dry weight (Appendix 6). At Elburgon site, the highest number of tubers and tuber dry weight were from the treatment F5 followed by F4 and the lowest was from control for all varieties (Table 5.2) same for semi-field experiment (Table 5.4). Whereas at Mau- Narok site, the highest number of tubers and tuber dry weight were from F4 followed by F5 and the lowest from control for all varieties (Table 5.3).

Table 5.2: Effect of canola green manure and fertilizer treatments on potato tuber yields (Elburgon site)

Fertilizer levels (kg ha^{-1})	*Shangi* variety		*Kenya Karibu* variety	
	Number of tubers per plant	Tuber dry weight (t ha^{-1})	Number of tubers per plant	Tuber dry weight (t ha^{-1})
NPK900 (F1)	8.07^{ab}	7.85^{ab}	7.18^{a}	5.62^{a}
NPK575 (F3)	7.77^{ab}	5.73^{bc}	6.33^{ab}	4.77^{ab}
DAP500+CAN300 (F5)	8.87^{a}	8.45^{a}	8.06^{a}	6.00^{a}
NPK250 (F2)	6.22^{bc}	4.82^{c}	5.62^{ab}	3.52^{bc}
Control (F1)	4.87^{c}	4.11^{c}	4.29^{b}	2.48^{c}
MSD	1.98	2.43	2.73	2.03
Green manure levels				
With green manure	7.31^{a}	6.50^{a}	6.77^{a}	4.85^{a}
Without green manure	7.01^{a}	5.88^{a}	5.82^{a}	4.11^{a}
MSD	3.70	7.29	4.13	4.28

The means followed by the same letters in the same column are not significantly different using Tukey’s honestly significant difference (HSD) test at P <0.05. ***MSD**: minimum significant difference.*

Table 5.3: Effect of canola green manure and fertilizer treatments on potato tuber yields (Mau-Narok site)

Fertilizer levels (kg ha^{-1})	*Shangi* variety		*Kenya Karibu* variety	
	Number of tubers per plant	Tuber dry weight (t ha^{-1})	Number of tubers per plant	Tuber dry weight (t ha^{-1})
NPK900 (F1)	11.90^{a}	7.85^{a}	10.20^{a}	11.42^{a}
NPK575 (F3)	12.37^{a}	6.20^{ab}	9.05^{ab}	8.94^{ab}
DAP500+CAN300 (F5)	11.33^{a}	6.29^{ab}	9.90^{a}	10.55^{a}
NPK250 (F2)	11.67^{a}	6.13^{ab}	8.15^{ab}	7.35^{b}
Control (F1)	8.83^{a}	4.57^{b}	6.62^{b}	6.71^{b}
MSD	5.81	2.95	2.47	6.06
Green manure levels				
With green manure	11.43^{a}	6.84^{a}	8.81^{a}	9.04^{a}
Without green manure	11.01^{a}	5.58^{a}	8.75^{a}	8.94^{a}
MSD	4.00	3.16	1.08	5.57

The means followed by the same letters in the same column are not significantly different using Tukey's honestly significant difference (HSD) test at P <0.05. ***MSD****: minimum significant difference.*

Table 5.4: Effect of canola green manure and fertilizer treatments on potato tuber yields (Semi-field experiment)

Fertilizer levels (kg ha^{-1})	Number of tubers per plant	Tuber dry weight (t ha^{-1})
NPK900 (F1)	12.33^{b}	2.14^{a}
NPK575 (F3)	11.33^{b}	1.82^{ab}
DAP500+CAN300 (F5)	17.92^{a}	2.45^{a}
NPK250 (F2)	9.92^{bc}	1.64^{ab}
Control (F1)	6.25^{c}	1.19^{b}
MSD	***5.43***	***0.81***

The means followed by the same letters in the same column are not significantly different using Tukey's honestly significant difference (HSD) test at P <0.05. ***MSD****: minimum significant difference.*

5.3.3 Potato tuber grade distribution across fertilizer and canola green manure treatments

Tuber grades were significantly influenced by potato varieties and fertilizer treatments (Appendix 7). Generally, the higher fertilizer treatment (F4) resulted in a higher percentage of big tubers (16.33%) while the negative control (F1) had significantly higher percentage of small size tubers. Canola green manure did not show a significant effect on tube size grades though, 18.68% increase of big size tuber grade was observed from canola green manure application over no green manure application under field conditions. Effect due to site differences were observed, Mau-Narok had high percentages of big tubers (16.03%) than Elburgon (9.00%) site. At Mau-Narok site, high percentages of big tubers 21.77%, and 16.00% were observed from the treatment treated with F4 followed with F5 respectively, where Elburgon had the high percentages of big tuber 11.30% and 12.08%, and were from F5 followed with F4 respectively. Generally, varietal differences were observed in tuber size distribution; *Kenya Karibu* variety had higher percentages of big tubers than *Shangi* but a significant difference were observed at Mau-Narok site (Appendix 8). The two treatments (F4 and F5) performed better across sites and varieties (Table 5.5 and 5.6**)**. For semi-field experiment, only *Shangi* variety was harvested and all the tubers were small in size.

Table 5.5: Effect of fertilizer and canola green manure on potato tuber grades at Mau-Narok site

Fertilizer levels (kg ha^{-1})	***Shangi* variety**			***Kenya Karibu* variety**		
	%Big size >60 mm	%Medium size 30-60 mm	%Small size <30 mm	%Big size >60 mm	%Medium size 30-60 mm	%Small size <30 mm
NPK900 (F1)	15.00^{a}	19.66^{a}	65.34^{a}	28.53^{a}	24.69^{ab}	46.79^{b}
NPK575 (F3)	7.30^{a}	16.78^{a}	75.79^{a}	20.10^{a}	27.69^{a}	52.21^{ab}
DAP500+CAN300 (F5)	9.38^{a}	18.15^{a}	72.46^{a}	22.62^{a}	21.62^{ab}	54.14^{ab}
NPK250 (F2)	8.30^{a}	20.83^{a}	70.84^{a}	21.27^{a}	18.98^{ab}	59.79^{ab}
Control (F1)	7.08^{a}	14.87^{a}	78.08^{a}	20.72^{a}	17.08^{b}	62.22^{a}
MSD	8.54	12.87	13.51	11.36	9.80	13.06
Green manure levels						
With green manure	10.34^{a}	20.77^{a}	68.86^{a}	23.01^{a}	22.00^{a}	54.34^{a}
Without green manure	8.49^{a}	15.35^{a}	76.15^{a}	22.28^{a}	22.02^{a}	55.72^{a}
MSD	13.32	23.28	27.21	7.68	7.01	3.04

The means followed by the same letters in the same column are not significantly different using Tukey's honestly significant difference (HSD) test at P<0.05. *MSD: minimum significant difference.*

Table 5.6: Effect of fertilizer and canola green manure on potato tuber grades at Elburgon site

Fertilizer levels (kg ha^{-1})	***Shangi* variety**			***Kenya Karibu* variety**		
	%Big size >60 mm	%Medium size 30-60 mm	%Small size <30 mm	%Big size >60 mm	%Medium size 30-60 mm	%Small size <30 mm
NPK900 (F1)	12.56[a]	26.88[a]	60.57[a]	10.04[a]	21.49[a]	68.47[a]
NPK575 (F3)	4.34[b]	25.87[a]	69.76[a]	9.49[a]	28.36[a]	62.15[a]
DAP500+CAN300 (F5)	12.86[a]	24.57[a]	62.58[a]	11.30[a]	29.12[a]	59.58[a]
NPK250 (F2)	8.72[ab]	25.87[a]	65.42[a]	6.68[a]	23.45[a]	69.87[a]
Control (F1)	6.91[ab]	27.50[a]	65.58[a]	7.11[a]	18.56[a]	74.32[a]
MSD	8.12	14.87	17.51	7.67	16.86	16.84
Green manure levels						
With green manure	10.37[a]	26.52[a]	63.11[a]	10.62[a]	25.16[a]	64.23[a]
Without green manure	7.79[a]	25.76[a]	66.46[a]	7.23[a]	23.23[b]	69.53[a]
MSD	25.79	21.14	42.12	18.19	28.69	44.83

The means followed by the same letters in the same column are not significantly different using Tukey's honestly significant difference (HSD) test at P <0.05. *MSD*: minimum significant difference.

5.3.4 Correlation between growth and yield parameters for field experiment

Growth parameters were positively correlated with yield parameters under field experiment Plant height was observed to be significantly positively correlated with yield parameters (number of tubers and tuber dry weight). Number of stems had a strong positive correlation with number tubers, but fairly correlated with tuber weight (Table 5.7).

Table 5.7: Pearson correlation coefficients (p-values) of growth and yield parameters for field experiment (combined for the two sites)

	Number of stems	Plant height	Number of tubers
Plant height	0.61 (<.0001)		
Number of tubers	0.56 (<.0001)	0.62 (<.0001)	
Dry tuber weight	0.06(0.54)	0.31(0.0005)	0.63(<.0001)

Effect of canola and fertilizer on nutrient uptake and use efficiency

Nitrogen (N), phosphorus and potassium (P) uptake were significantly affected by selected fertilizer treatments. Nutrient uptake was higher with F4 and F5 treatments than control. The highest nutrient uptake of 139.07 kg ha^{-1}, 10.58 kg ha^{-1} and 119.26 kg ha^{-1} (N, P and K) by potato tubers was recorded where F4 fertilizer treatments were applied. Generally, F4 increased nutrients (N, P and K) uptake by 13%, 25% and 3% respectively over F5 under field experiment. Nutrients (N, P and K) uptakes were not significantly different between potato varieties. Site differences exhibited differences on nutrients uptake, the plant at Mau-Narok showed higher 132.58 kg ha^{-1}, 10.72 kg ha^{-1} and 121.95 kg ha^{-1} (N, P and K) than Elburgon 90.67 kg ha^{-1}, 5.12 kg ha^{-1}, 72.52 kg ha^{-1} (N, P and K). Canola green manure increased nutrients (N, P and K) uptake by 24%, 21% and 11% over no green manure application under field experiment. The same trend was observed across the sites and semi-field experiment, where F4 and F5 treatments were not significantly different between them on nutrients uptake, but different from control (Table 5.8). Canola green manure had no significant effect across the sites and semi-field experiment.

Nitrogen recovery was significantly affected by selected fertilizer treatments (F4 and F5) but phosphorus was not significantly different on fertilizer treatments. Generally, F4 treatments increased nutrients recovery by 131% (N) and 55% (P) over F5. The same trends were observed across the sites and semi-field experiment that (N) was high with F4 treatment application (Table 5.9). Under semi-field experiment, nutrients recovery was significantly different between F4 and F5 fertilizer treatments. Fertilizer use efficiency was not significantly different among fertilizer treatments, and the same trend was observed under semi-field and field experiments (Table 5.10).

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able 5.8*:* Effect of selected fertilizer treatments on potato nutrients; nitrogen (N), phosphorus (P) and potassium (K) uptake for field and semi-field experiments

Fertilizer levels kg ha^{-1}	***Shangi* variety**			***Kenya Karibu* variety**		
	N (kgha^{-1})	P (kg ha^{-1})	K (kg ha^{-1})	N (kg ha^{-1})	P (kg ha^{-1})	K (kg ha^{-1})
			Mau-Narok			
NPK900 (F4)	157.53^{a}	13.99^{a}	134.69^{a}	165.34^{a}	15.79^{a}	182.61^{a}
DAP500+CAN300 (F5)	119.43^{ab}	8.06^{b}	102.92^{a}	163.08^{a}	12.53^{ab}	158.09^{a}
Control (F1)	89.57^{b}	6.89^{b}	80.10^{a}	100.54^{b}	7.06^{b}	73.31^{b}
MSD	*44.72*	*4.34*	*70.69*	*29.49*	*8.00*	*66.36*
			Elburgon			
NPK900 (F4)	140.65^{a}	5.82^{ab}	86.91^{ab}	92.76^{a}	6.72^{a}	72.84^{ab}
DAP500+CAN300 (F5)	120.44^{a}	9.13^{a}	115.96^{a}	90.99^{a}	4.00^{ab}	87.24^{a}
Control (F1)	63.52^{b}	3.60^{b}	49.87^{b}	35.69^{b}	1.43^{b}	22.32^{b}
MSD	*46.45*	*5.48*	*49.62*	*34.87*	*3.00*	*51.57*
			Semi-field experiment			
NPK900 (F4)	39.11^{a}	3.41^{a}	33.21^{ab}	-	-	-
DAP500+CAN300 (F5)	44.87^{a}	4.12^{a}	43.216^{a}	-	-	-
Control (F1)	19.65^{b}	1.84^{b}	14.37^{b}	-	-	-
MSD	*13.17*	*1.52*	*19.18*	-	-	-

The means followed by the same letters in the same column are not significantly different using Tukey's honestly significant difference (HSD) test at P <0.05. *MSD: minimum significant difference*. *Kenya Karibu* data for semi-field experiment are missing because it was stolen at harvesting stage.

Table 5.9: Effect of selected fertilizer treatments on potato nutrients; nitrogen (N) and phosphorus (P) recovery for field and Semi-field experiments

Fertilizer levels (kg ha^{-1})	*Shangi* variety		*Kenya Karibu* variety	
	N (%)	P (%)	N (%)	P (%)
	Mau-Narok			
NPK900 (F4)	75.51^a	3.04^a	72.00^a	3.73^a
DAP500+CAN300 (F5)	18.55^b	0.51^b	38.85^b	2.38^a
MSD	*42.05*	*1.98*	*30.40*	*4.24*
	Elburgon			
NPK900(F4)	83.75^a	0.95^a	63.42^a	2.26^a
DAP500+CAN300 (F5)	35.35^b	2.40^a	34.34^b	1.12^a
MSD	*41.99*	*2.75*	*27.88*	*1.41*
	Semi-field experiment			
NPK900 (F4)	21.63^a	0.67^a	-	-
DAP500+CAN300 (F5)	14.31^a	0.91^a	-	-
MSD	*12.62*	*0.73*	-	-

The means followed by the same letters in the same column are not significantly different using Tukey's honestly significant difference (HSD) test at P <0.05. ***MSD****: minimum significant difference*. *Kenya Karibu* data for semi-field experiment are missing because it was stolen at harvesting stage.

Table 5.10: Effect of fertilizer treatments on potato fertilizer use efficiency (Field and Semi-field experiments)

Fertilizer levels (kg ha^{-1})	Field experiment (kg ha^{-1})	Semi-field experiment (kg ha^{-1})
NPK900 (F4)	4.19^a	1.06^a
DAP500+CAN300 (F5)	4.19^a	1.10^a
NPK575 (F3)	3.38^a	1.57^a
NPK250 (F2)	3.95^a	1.82^a
MSD	*3.54*	*2.69*

The means followed by the same letters in the same column are not significantly different using Tukey's honestly significant difference (HSD) test at P <0.05. ***MSD****: minimum significant difference*.

5.4 Discussion

5.4.1 Potato growth response to fertilizer and canola green manure application

Generally, fertilizer application corresponds to increased plant growth and yield, but care should be taken when choosing the type of fertilizer to apply depending on soil and crop requirement. Potato is a heavy feeder, shallow rooted and it requires a wide range of nutrients to realize full potential yield (Westermann, 2005). Assessment of fertilizer application success is based on crop growth and yield. In this study, fertilizer treatments did not affect the number of stems. Adhikari (2009) reported the same, on the study that was evaluating the effected of NPK fertilizer on growth and yield of potato, he found that fertilizer had no significant effect on number of stems. Number of stems may depend on variety character, number of eyes on seed tubers and pre-sprouting treatments instead of nutrients from the soil. Wurr *et al*. (2001) found that the number of stems were associated with environmental conditions at the time of tuber initiation of the seed crop and sprouting temperature. Nielson *et al.* (1989) reported that number of potato tuber eyes determine number of stems, tuber set and yield of potato cultivar; he also noticed a strong correlation coefficient between the number of eyes on potato seed tuber and number of stems produced. Bohl *et al.* (*2000*) reported that number of eyes per potato tuber seed influences stems number. He reported also that every eye has a potential to produce at least one stem under good physiological condition. Adrien (2013) observed that, number of shoots per plant depends mainly on genetic make-up of the variety, development phases of sprouts at planting time, grade (size) and the number of eyes of mother-tuber.

Although, fertilizer treatments enhanced potato plant height and (NPK+Ca+Mg+) applied at rate of 900 kg ha^{-1} (F4) resulted in the highest plant height, generally the higher the rate of fertilizer application the higher the plant height. This may be attributed to the high amount of phosphorus that enhanced root development, and improved nutrients uptake, high nitrogen that enhanced vegetative growth, good canopy cover and photosynthesis. Nitrogen (N) is one of the most crucial macronutrients for plant growth and biomass development reported by Koch *et al*. (2019). Adhikari (2009) in the study that was evaluating effect of different nutrient levels (0:0:0, 50:50:50, 100:50:50, 100:75:50, 100:75:100, 100:100:100 and 150:100:100 N, P_2O_5 and K_2O kg ha^{-1}) on potato vegetative growth and yield, found that plant height was significantly affected by different levels of fertilizer application and increased by 15-42% as compared to the control. He concluded that it was due to high dose of nitrogen (N) resulting in vigorous plant growth. The same trend was observed by Nizamuddin *et al*. (2003), who found that, plant height (cm) increased with increased nitrogen application 54.37 cm when 150 kg N ha^{-1} was applied and 59.33 cm with 200 kg N ha^{-1}application.

5.4.2 Potato yield response to fertilizer treatments and canola green manure application

Fertilizer treatments also had effect on potato tuber dry weight and number of tubers, the results shows that the two treatments (NPK+Ca+Mg+) applied at rate of 900 kg ha^{-1} (F4) and DAP500 kg ha^{-1} (F5) were the best performers in all sites and semi-field experiment. These two treatments were not significantly different either for number of tuber or tuber dry weight as for growth parameters. Though there was an increase of 4.73% and 83.22% tuber dry weight from NPK 900 kg ha^{-1} fertilizer treatment over positive control DAP500 kg ha^{-1} and negative control respectively under field condition and this may be due to additional nutrients in NPK over DAP. Normally potato requires adequate amounts of primary macronutrients (N, P, and K) and secondary macronutrients (Ca and Mg) to grow well and gives high tuber yields (Haifa, 2020; Koch *et al*., 2019). The available potato recommendation in Kenya does not cater for potassium (K) due to believing that Kenyan soils have enough potassium (Kanyanjua & Ayaga, 2006; Muthoni & Nyamongo, 2009). Potassium is a key nutrient that is needed throughout potato growing period. Potassium in potato is critical for increased yield; it influences the transport of nutrients and the movement of sucrose from the leaf to the tuber. In five trials across three years on K-rich volcanic soils found that potassium (K_2O) applied at a rate of 120 kg ha^{-1} increased average yields by 10 t ha^{-1} (YARA, 2020). Magnesium and calcium nutrients are not also considered in nutrients recommendation yet they have a great influence on potato yield and soil fertility maintenance. Magnesium enhances crop performance and yield through improved physiological process in plant (Wang *et al*., 2020). Magnesium plays an important role in photosynthesis and involves in translocation of sugars from leaves to the potato tubers and production of sugars and proteins. Magnesium is mostly need during potato bulking stage (YARA, 2020). Calcium is an important building block of cell wall and stability of membranes, it also acts as a bridge in transport of the phosphate and carboxylate groups of phospholipids and proteins at the membrane surfaces (Koch *et al*., 2019). The study conducted by Nesreen *et al*. (2015) showed that Ca and K fertilizers applied at 178.57 (K) kg ha^{-1} and 47.62 (Ca) kg ha^{-1} enhanced potato growth and yield. A three years study conducted by Talukder *et al*. (2009) found that magnesium had significant effects on tuber yield of potato, treatment of 10 (Mg) kg ha^{-1} had higher tuber yield of 32.33, 31.63, and 28.03 t ha^{-1} in three successive years.

Potato crop respond differently under different soil and agro-ecological zones. In this study**,** site differences were observed for growth and yield parameters where they were higher in Mau- Narok than Elburgon. Initially all sites had adequate N and K with low amount of phosphorus (P), and calcium (Ca) at Elburgon. Mau-Narok and Egerton sites had good soil pH ideal for potato production 5.68 and 5.47 respectively, whereas Elburgon site had a strong acidic soil condition (pH 4.57) that was not ideal for potato production. Potato grows well in pH range of 5.5 to 7 with ideal of 5.5 (NPCK, 2019). Soil pH is key factor affecting biogeochemical processes in the soil. Low pH is mostly as a result excessive leaching of basic cations (Ca, Mg, K, and Na), leaving H^+ and Al^{3+} ions (Neina, 2019). Too acidic soil affects availability of nutrients to plant roots, either by fixation and/or by toxification. In addition, calcium (Ca) deficiency interferes with root growth, causes deformation of foliage growth tips, and may result in reduced and poor quality yields as reported by Haifa (2020).

There are many potato varieties grown in Kenya**,** all differ in genetic, morphology, growth habit, maturity, tuber dormancy period, yield potential, resistance to biotic and abiotic stresses and soil and climatic requirement (NPCK, 2019). In this study, two potato varieties were used and they showed a significant difference on growth and yield parameters. *Shangi* had high number of stems, plants height and high number of tubers per plant, though on tuber dry weight and percentage of big size tubers, *Kenya Karibu* had higher values. *Shangi* was susceptible to potato bacterial wilt disease and heavy rains stress compared to *Kenya Karibu.* The differences in performance may also be attributed to genetic makeup difference and growth nature of the two varieties. *Kenya Karibu* takes long time to emerge, flower and mature compared to *shangi* variety. These findings are supported by Koch (2019) who stated that, genetic background determine the yield potential of the potato. Tsegaw (2011) on the study that was evaluating genotype by environment interaction for tuber yield, dry matter content and specific gravity for (11) potato genotypes, reported significant variations among potato genotypes with respect to tuber yields and dry matter content. The same observation was reported by Tapiwa (2016) that, a significant difference in the yields was due to differences in genetic makeup of potato varieties.

5.4.3 Pearson correlation coefficient analysis between growth and yield parameters

Mostly**,** vegetative growth determines the potato tubers to be produced, in this line Pearson correlation coefficient analysis showed that, plant height was positively correlated with number of tubers and tuber dry weight. This means that, the higher potato growth the more the number of tubers and tuber weight. Number of stems were strongly correlated to number of tubers, but fairly correlated with tuber weight, this means, the more the number of stems the more the number of tubers and tuber weight. These results are supported by Adhikari, (2009) who revealed that, the yield increase of potato tuber was associated with increase in the plant height, fresh weight of leaves and stems as a result of applied NPK. The same result was observed by Burhan (2007) who found a strong positive correlation between tuber yield and plant height (r = 0.745) and was significant.

5.4.4 Potato nutrients use efficiency (NUE) response to fertilizer and canola green manure application

Nutrient use efficiency (NUE) is one of the tools used to measure efficacy of soil supplied nutrients and is defined as a measure of how well plants use the available mineral nutrients. NUE depends on the ability of the plants to take up and utilize the nutrients available in the soil, and the ability of the soil to supply all necessary condition for the plants to take up the available nutrients (Hawkesford *et al*., 2014). One way of supplying nutrients to the plants is application of inorganic fertilizers. Fertilizer use efficiency may be affected by many factors such as; type of crop, soil properties, climatic condition, forms of fertilizer (NH_4^+-N or NO_3 -N), soil microorganisms such as mycorrhiza, and others (Baligar *et al*., 2001). Nitrogen form (NH_4^+ or NO_3^-) has an important effect on potato nitrogen uptake and use efficiency (Petropoulos, *et al* 2020). In this study (NPK+Ca+Mg+) showed increased nutrients (N, P and K) uptake and nutrients recovery over diammonium phosphate (DAP) and this may be due to additional nutrients in (NPK+Ca+Mg+) fertilizer over DAP that enhanced other nutrients uptake. Calcium (Ca) and magnesium (Mg) improves soil property and nutrients availability through improved soil pH and cation exchange capacity (CEC). Hazleton and Murphy (2007) reported that, Cation exchange capacity (CEC) is a very important soil property that influences physical and chemical properties such as; soil structure stability, nutrient availability, soil pH as well as the soil's ability to react with soil amendments (fertilizers). In this study, (NPK+Ca+Mg+) fertilizer increased potato yield. Rosen (2020) reported that yield is closely related to potato nutrients uptake, where double the yield will result in twice nutrients removal. Ammonium based fertilizers such as DAP are one of nitrogen (N) source, however they gradually acidify soil through release of H^+ in the process of converting ammonium to nitrate by soil bacteria (Crop Nutrition, 2020). Moreover, low soil pH may lead to Mn and aluminum (Al) toxicity and P, K, Ca, and Mg deficiencies (Rosen, 2020). Through use of fertilizer best management practices, which includes application of right source of nutrients at the right rate, time, and place fertilizer maybe used efficiently (Terry, 2007).

5.5 Conclusion

The study indicated that fertilizer (NPK+Ca+Mg+) applied at rate of 900 kg ha^{-1} increased potato plant height, nutrient uptake and potato yield over DAP and negative control. (NPK+Ca+Mg+) has additional nutrients that are required by potato for better growth and production, and has not been shown to have any negative effect on soil acidification as reported on continuous use of DAP. Generally, canola green manure did not show a significant effect on potato plant height and yield; although F4 with green manure application exhibited an increase of 7% and 38% on plant height and tuber dry weight respectively over no green manure application. The study recommends the use of (NPK+Ca+Mg+) applied at rate of 900 kg ha^{-1} for potato production in Kenya upon economic evaluation. The study further recommends additional research to assess compound fertilizers over several seasons with monitoring and evaluation of their effect on soil physical and chemical properties.

CHAPTER SIX

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

6.1 General discussion

Proper selection and use of agricultural inputs including fertilizers can increase potato production in Kenya. The results from this study showed that (NPK+Mg+Ca+) applied at a rate of 900 kg ha^{-1}(F4) increased potato plant height and tuber weight compared to DAP and control. For maximum production, potato require balanced nutrient application that include nitrogen (N), phosphorus (P), potassium (K), magnesium (Mg) and calcium (Ca) (Haifa, 2020; Koch *et al*., 2019). The available potato recommendation in Kenya does not cater for potassium (K) (Kanyanjua & Agaya, 2006; Muthoni & Nyamongo, 2009), magnesium and calcium as major nutrients. Potassium influences the transport of nutrients and the movement of sucrose from the leaf to the tuber. Magnesium plays an important role in photosynthesis and is involved in translocation of sugars from leaves to the potato tubers and production of sugars and proteins (Crop nutrition, 2020; Wang *et al*., 2020). Other studies have shown that potassium (Crop Nutrition, 2020), magnesium (Hoyum, 2000) and calcium (Nesreen *et al*., 2015) applications increased potato yields.

Furthermore, (NPK+Mg+Ca+) enhanced nutrients use efficiency compared to Diammonium phosphate (DAP). This may be due to the synergic effect of additional nutrients in NPK over DAP. Continuous application of ammonium-based fertilizer can gradually acidify soil (Muthoni *et al*, 2016) and acidic soil may affect nutrient use efficiency. In Kenya, around 96% of potato farmer's use DAP, 1% use NPK (Kaguongo *et al.,* 2008) and the research shows that in Western and Central Provinces, 7.5 million hectares of land are highly acidic (Kanyanjua, 2002). The most common method of managing soil acidity is application of lime mostly based on calcium and magnesium, which increase cost of production. Application of Mg and Ca based fertilizer can have a great impact on soil improvement. Calcium helps to maintain chemical balance in the soil, reduces soil salinity, and improves water penetration (Tetra chemicals, 2020). Calcium helps in aggregating soil particles hence improving soil structure and stabilizing organic matter, which increases soil water- and nutrient-holding capacity (Midwestern bioag, 2020).

In this study, the main purpose of canola green manure was management of potato bacterial wilt. Canola green manure did not affect *R. solanacearum population* density in the soil and bacterial wilt incidence. It has been reported that brassica plants have biofumigation potential in suppressing soil borne pathogens through release of glucosinolates compounds

(Matthiessen & Kirkegard, 2006). Glucosinolates are sulfur-containing compounds that are released by plants through plant cell rupture, and produce active compound called isothiocyanates through hydrolysis process in the presence of enzymes (Flamini, 2012). There is limited information on the effect of brassicas on soil borne bacteria. Moreover, Larkin and Griffin (2007) found highest inhibition of most of fungal pathogens assessed (*F. Oxysporum, R. solani, Phytophthora erythroseptica, and Pythium ultimumfrom*) due to Indian mustard application under *in-vitro* experiment. While under field experiment, Indian mustard was most effective for reducing powdery scab and common scab diseases, rapeseed and canola were most effective in reducing *Rhizoctonia* diseases.

Potato varieties has been found to respond differently to potato bacterial wilt disease, *Kenya Karibu* did not show any symptoms of bacterial wilt whereas *Shangi* was most severely affected. There is no potato variety that has been found to be resistant to bacterial wilt 100%, but some level of tolerance may be observed (Felix *et al*., 2010; NPCK, 2019). Even if no symptoms are observed, tolerant varieties carry latent infection to the next progeny once planted as seeds (Priou *et al.,* 1996). Felix *et al*. (2010) and Muthoni *et al*. (2014b) revealed that *Kenya Karibu* potato variety have some level of tolerance to bacterial wilt.

6.2 General conclusions

In this study application of (NPK+Mg+Ca+) at the rate of 900 kg ha^{-1} (F4) increased potato plant height by 5.0%, tuber dry weight by 5.0% and nutrients (nitrogen, phosphorus and potassium) uptake by 13%, 26% and 3% respectively compared to use of DAP and CAN applied at 500 and 300 kg ha^{-1} respectively. Generally, canola green manure did not show a significant effect on potato plant height and yield, though F4 with green manure exhibited an increase of 7% and 38% on plant height and tuber dry weight respectively. Canola, extract did show any effect in reduction of *Ralstonia solanacearum* population density *in vitro,* it seemed to enhance growth of pathogen under laboratory conditions. Canola green manure also did not show effect on potato bacterial wilt incidence nor *Ralstonia solanacearum* population density reduction in the soil under field experiments. *Kenya Karibu* did not show wilting symptoms of bacterial wilt across the sites and semi-field experiment.

6.3 Recommendations

a) Further research should be done on other locally available brassica plants by determining those with high glucosinolates contents and their potential to control *R. solanacearum*.

b) *Kenya Karibu* variety showed to be tolerant in the study sites, further screening for resistance of other varieties available in Kenya should be done.

c) The study showed that (NPK+Mg+Ca+) is beneficial in potato production, more investigation should be done under none *Ralstonia solanacearum* soils on its effect on potato yield and soil properties over time in consideration of economic analysis.

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APPENDICES

Appendix 1: Combined analysis of variance of growth parameters (Mau-Narok and Elburgon sites)

Source of variation	df	Number of stem28 DAE	Plant height (cm) 28DAE	Number of stem35 DAE	Plant height (cm) 35DAE	Number of stems 42DAE	Plant height (cm) 42DAE	Plant height (cm) 56DAE
Site	1	12.03**	2286.39***	15.41**	5216.65***	13.33***	7113.26***	908.60*
Gm	1	0.13	6.82	0.08	2.13	0.13	18.80	155.95
Site×gm	1	0.53	2.41	0.08	0.53	0.00	0.04	42.72
Main error	6	0.47	23.89	0.60	62.53	0.35	60.38	179.00
Fert	4	0.60	46.44***	0.36	204.94***	0.59	504.50***	1516.99***
Fert×gm	4	0.74	0.86	0.97	18.47	0.86	41.89	36.38
Site×gm×fert	4	0.22	2.41	0.30	7.65	0.29	7.64	44.31
Var	1	26.13***	9268.18***	29.01***	15627.41***	32.03***	19530.46***	10486.96***
Var×fert	4	0.57	7.09	0.57	44.21**	0.53	39.26**	34.43
Var×fert×site	4	0.49	4.08	0.30	3.20	0.09	32.42	18.87
Var×gm	1	0.30	3.27	0.68	0.50	0.83	1.34	15.27
Site×gm×var	1	0.30	3.82	1.00	0.36	0.83	0.33	0.07
Gm×var×fert	4	0.11	11.21	0.36	14.29	0.29	21.18	44.51
Site×gm×var×fert	4	0.45	4.01	0.11	8.97	0.25	33.51	78.25
Cv		***20.15***	***13.81***	***20.02***	***11.15***	***21.01***	***11.28***	***13.66***

*, **, *** significant at (p=0.05); (p=0.01), (p=0.001) respectively. ***Gm****:* canola green manure, ***var****:* variety, ***fert****:* fertilizer*,* ***Cv****:* Coefficient of variation, ***DAE****:* days after emergence.

Appendix 2: Analysis of variance for growth and yield parameters (Simi-field experiment)

Source of variation	**df**	**Number of stem28 DAE**	**Plant height (cm) 28 DAE**	**Number of stem35 DAE**	**Plant height (cm) 35 DAE**	**Number of stems 42 DAE**	**Plant height (cm) 42 DAE**	**Plant height (cm) 56DAE**	**Number of tubers per plant**	**Tuber dry weight per plant (t ha^{-1})**
Gm	3	0.25	0.61	0.03	0.59	0.07	0.65	0.27	0.50	0.06
Fert	4	0.67	0.92	0.63	8.15***	0.52	9.77***	13.12***	4.63***	0.41***
Gm×fert	12	0.17	0.24	0.18	0.37	0.17	0.32	0.47*	0.39	0.04
Var	1	9.10***	150.66***	8.89***	39.79***	9.20***	24.09***	13.43***	-	-
Var×gm	3	0.77	0.15	0.74*	0.22	0.71*	0.08	0.12	-	-
Var×fert	4	0.06	0.47	0.05	1.28	0.06	1.15*	1.16**	-	-
Var×fert×gm	12	0.21	0.33	0.22	0.36	0.25	0.33	0.19	-	-
Cv		***21.97***	***16.71***	***22.09***	***12.04***	***21.76***	***10.4***	***15.03***	***20.65***	***20.49***

All data were transformed using square root. *, **, *** significant at (p=0.05); (p=0.01), (p=0.001) respectively. ***Gm**:* canola green manure, ***var**:* variety, ***fert**:* fertilizer, ***Cv**:* Coefficient of variation, ***DAE**:* days after emergence.

Appendix 3: Analysis of variance for growth parameters at Mau-Narok site

Source of variation	df	Number of stem28 DAE	Plant height (cm) 28DAE	Number of stem35 DAE	Plant height (cm) 35DAE	Number of stems 42DAE	Plant height (cm) 42DAE	Plant height (cm) 56DAE
Gm	1	0.07	0.56	0.00	2.40	0.07	8.51	17.71
Main error	2	0.17	8.00	0.00	19.75	0.07	34.34	13.53
Fert	4	0.65	36.11**	0.68	134.98***	0.78	258.97***	473.35***
Gm×fert	4	0.57	2.93	1.00	13.52	0.86	42.35**	52.94
Var	1	52.27***	10613.40***	56.07***	17129.84***	56.07***	17922.82***	10433.29***
Var×fert	4	0.10	7.80	0.07	21.52	0.11	2.92	11.25
Gm×var	1	0.60	0.01	1.67	0.86	1.67	0.17	8.66
Gm×var×fert	4	0.18	14.02	0.08	22.83	0.13	46.47	69.24
Cv		***19.55***	***12.55***	***18.82***	***9.42***	***19.67***	***6.71***	***8.71***

*, **, *** significant at (p=0.05); (p=0.01), (p=0.001) respectively. ***Gm:*** canola green manure, ***var:*** variety, ***fert:*** fertilizer, ***Cv:*** Coefficient of variation, ***DAE:*** days after emergence.

Appendix 4: Analysis of variance for growth parameters at Elburgon site

Source of variation	df	Number of stem28 DAE	Plant height (cm) 28DAE	Number of stem35 DAE	Plant height (cm) 35DAE	Number of stems 42DAE	Plant height (cm) 42DAE	Plant height (cm) 56DAE
Gm	1	0.60	8.66	0.15	0.27	0.07	10.33	180.96
Main error	2	0.65	54.29	0.65	110.99	0.17	137.07	520.56
Fert	4	0.21	14.31*	0.19	82.27***	0.48	253.82***	1181.29***
Gm×fert	4	0.39	0.34	0.28	2.61	0.32	7.17	27.73
Var	1	0.00	1097.39***	0.02	2107.52***	0.27	4065.62***	1821.61***
Var×fert	4	0.96*	3.38	0.81	25.89	0.52	68.77*	42.04
Gm×var	1	0.00	7.07	0.02	0.01	0.00	1.50	6.67
Gm×var×fert	4	0.38	1.21	0.39	0.42	0.42	8.22	53.52
Cv		***20.77***	***15.52***	***21.47***	***13.90***	***22.61***	***17.55***	***17.94***

*, **, *** significant at (p=0.05); (p=0.01), (p=0.001) respectively. ***Gm****:* canola green manure, ***var****:* variety, ***fert****:* fertilizer, ***Cv****:* Coefficient of variation, ***DAE****:* days after emergence.

Appendix 5: Combined analysis of variance for yield parameters (Mau-Narok and Elburgon sites)

Source of variation	Df	Number of tubers per plant	Tuber dry weight per plant (t ha^{-1})
Site	1	321.80^{**}	153.84^{**}
Gm	1	5.66	13.99
Site×gm	1	1.10	0.00
Main error	6	10.76	11.14
Fert	4	46.05^{***}	58.93^{***}
Fert×gm	4	6.20	15.24^{***}
Site×fert	4	2.95	2.46
Site×gm×fert	4	12.24^{*}	12.27^{***}
Var	1	81.59^{***}	8.60^{*}
Var×fert	4	1.75	1.05
Var×fert×site	4	1.27	4.33
Var×gm	1	0.15	1.99
Site×gm×var	1	1.93	3.10
Gm×var×fert	4	4.03	7.69^{*}
Site×gm×var×fert	4	1.72	2.31
Cv		***23.74***	***23.31***

*, **, *** significant at ($p \leq 0.05$); ($p \leq .01$), ($p \leq 0.001$) respectively. ***Gm****:* canola green manure, ***var****:* variety, ***fert****:* fertilizer, ***Cv****:* Coefficient of variation, ***DAE****:* days after emergence.

Appendix 6: Analysis of variance for yield parameters at Mau-Narok and Elburgon sites

Source of variation		**Elburgon**		**Mau-Narok**	
	df	Number of tubers per plant	Tuber dry weight per plant (t ha^{-1})	Number of tubers per plant	Tuber dry weight per plant (t ha^{-1})
Gm	1	5.87	6.99	0.89	7.00
Main error	2	12.29	22.70	19.80	9.14
Fert	4	27.49***	33.17***	21.51*	28.22***
Gm×fert	4	3.87	1.46	14.58	26.06***
Var	1	11.14*	44.03***	89.06***	116.29***
Var×fert	4	0.37	1.16	2.65	4.21
Gm×var	1	1.58	0.06	0.50	5.03
Gm×var×fert	4	2.74	3.05	3.01	6.96*
Cv		***20.93***	***26.19***	***24.29***	***21.18***

*, **, *** significant at (p≤0.05); (p≤0.01), (p≤0.001) respectively. ***Gm****:* canola green manure, ***var****:* variety, ***fert****:* fertilizer, ***Cv****:* Coefficient of variation, ***DAE****:* days after emergence.

Appendix 7: Combined analysis of variance for tuber grades (Mau-Narok and Elburgon)

Source of variation	**Df**	**%Big size >60mm**	**%Medium size 30 60mm**	**%Small size<30mm**
Site	1	26.54*	8.38	128.28
Gm	1	2.26	2.08	563.12
Site×gm	1	1.20	0.00	0.00
Main error	6	2.68	4.61	581.92
Fert	4	3.89***	2.07	345.52*
Fert×gm	4	1.33	0.21	58.23
Site×fert	4	0.64	0.18	96.61
Site×gm×fert	4	1.26	2.91*	443.12**
Var	1	24.76***	0.44	1774.01***
Var×fert	4	0.68	1.06	170.15
Varvfert×site	4	0.34	0.86	45.12
Var×gm	1	0.04	0.01	29.33
Site×gm×var	1	0.02	1.81	116.05
Gm×var×fert	4	1.79	1.28	110.08
Site×gm×var×fert	4	0.68	0.13	59.99
Cv		*24.86*	*20.74*	*15.53*
Grand mean		*12.52*	*22.60*	*64.80*

The data on big size and medium size tubers were transformed using square root. *, **, *** significant at at ($p \leq 0.05$); ($p \leq .01$), ($p \leq 0.001$) respectively. ***Gm****:* canola green manure, ***var****:* variety, ***fert****:* fertilizer, ***Cv****:* Coefficient of variation, ***DAE****:* days after emergence.

Appendix 8: Analysis of variance for tuber grades at Mau-Narok and Elburgon sites

Source of variation		**Elburgon**			**Mau-Narok**		
	Df	%Big size >60mm	%Medium size 30-60mm	%Small size <30mm	%Big size >60mm	%Medium size 30-60mm	%Small size <30mm
Gm	1	3.38	0.81	280.97	0.08	1.14	282.14
Main error	2	6.21	3.52	772.47	1.48	2.42	120.92
Fert	4	2.64*	0.68	133.79	1.89*	1.11	308.33
Gm×fert	4	0.56	1.69	22.81	2.03*	0.92	275.54
Var	1	0.04	1.31	65.73	46.80***	4.19*	4579.59***
Var×fert	4	0.95	1.06	153.01	0.07	0.60	62.26
Gm×var	1	0.06	0.57	14.35	0.00	1.16	131.04
Gm×var×fert	4	0.26	0.61	39.34	2.22*	0.46	130.72
Cv		*31.05*	*19.12*	*17.95*	*20.00*	*18.38*	*12.43*
Grand mean		*9.00*	*25.17*	*65.83*	*16.03*	*20.03*	*63.76*

The data on big size and medium size tubers were transformed using square root. *, **, *** significant at at (p≤0.05); (p≤.01), (p≤0.001) respectively. ***Gm:*** canola green manure, ***var:*** variety, ***fert:*** fertilizer, ***Cv:*** Coefficient of variation, ***DAE:*** days after emergence.

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Appendix 10: Published article on the effect of compound fertilizer and canola green manure on nutrient use efficiency, growth and yield of potato tuber (*Solanum tuberosum* L.) in Nakuru, Kenya

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PLANT NUTRITION | ORIGINAL ARTICLE

Effects of compound fertilizer and canola green manure on nutrient use efficiency, growth and yield of potato tuber (*Solanum tuberosum* L.) in Nakuru, Kenya

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ABSTRACT

Unbalanced fertilization is a problem affecting potato production in Kenya, where continuous use of nitrogen-phosphorus fertilizer (diammonium phosphate: DAP) has led to depletion of other macro and micronutrients. Hence, the need to assess alternative soil amendments including use of multi-nutrients compound fertilizer and canola green manure in potato production. Field and pot experiments were conducted in Nakuru during the period from August-December 2019 using two potato varieties (Shangi and Kenya Karibu) in randomized complete block design (RCBD) in split plot arrangement replicated three times (variety as main plot and combination of fertilizer and canola green manure as main plot). Two canola green manure levels (with and without), four levels of fertilizer (NPK + Ca + Mg + micronutrients) at 0 (F1), 250 (F2), 575 (F3), 900 (F4) kg ha^{-1} and recommended fertilizer rate (DAP at 500 kg ha^{-1} + Calcium ammonium nitrate (CAN300 kg ha^{-1}), (F5) as a positive control were used. Pot experiment was carried out at Egerton university farm in a completely randomized design (CRD) with three replicates. Four levels of canola green manure (100, 75, 50 and 0 g kg^{-1} soil) and five levels of fertilizer (NPK + Ca + Mg + micronutrients) as used in the field experiment were used. The results indicated that fertilizer F4 increased potato tuber dry weight and plant height by 5.0 and 5.0%, respectively over the normal recommended F5 under field experiments. F4 also increased nutrients (nitrogen, phosphorus and potassium) uptake by 13, 26 and 3%, respectively under field experiment compared to F5. Generally, canola green manure did not show a significant effect on plant height and yield, though F4 with green manure exhibited an increase of 7 and 38% on plant height and tuber dry weight, respectively. The study recommends the use of F4 (900 kg ha^{-1} of NPK + Ca + Mg + micronutrients) for potato production in Kenya and further recommends additional research to assess compound fertilizers over more seasons with monitoring and evaluation of their effect on soil physical and chemical properties and their economic feasibility.

Keywords: Potato nutrition, diammonium phosphate, multi-nutrients fertilizer

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1 Introduction

Potato (*Solanum tuberosum* L.) is a staple food in Kenya, and approximately 800,000 farmers are involved in the production generating about 500 million USD annually (AGRA, 2019). Kenya is among the top potato producing countries in Eastern Africa community with large production area, though potato production per hectare is declining year by year (Fig. 1) (FAOSTAT, 2020). Despite its importance, the pro-

Appendix 11: Published article on the effect of canola and compound fertilizer on potato (*Solanum Tuberosum* L.) Bacterial wilt management

ORIGINAL ARTICLE

European Journal of Agriculture and Food Sciences
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Effect of Canola and Compound Fertilizer on Potato (*Solanum Tuberosum* L.) Bacterial Wilt Management

Norbert Iraboneye, Miriam K. Charimbu, and Nancy W. Mungai

ABSTRACT

Bacterial wilt is a problematic disease affecting potato production in Kenya and the available management methods are not efficient. Field, semi-field and laboratory experiments were conducted to evaluate the effect of canola green manure and compound fertilizer on bacterial wilt management. Laboratory experiment was conducted at Egerton university biological laboratory to evaluate the effect of canola extract on *R. solanacearum* population density in-vitro. Four levels of canola extract quantities were used; 0, 100, 200, 300microlitres, and enrich immunomodulator (Di-bromo Di-nitro propane 1-3-diol) (DDD) was used as a positive control. Inoculum was prepared from infested soil and Selective Medium South Africa (SMSA) (Casamino acids, Bacto-Peptone, Glycerol, and Bacto-Agar) was used as growth medium in a completely randomized design. Field experiment was carried out in two sites (Elburgon site is in Upper high land zone two (UH2) and Mau-Narok is in Upper high land zone one UH1). Two levels of canola green manure (with and without green manure application), four levels of fertilizer (NPK+Ca+Mg+ micronutrients) applied at 0(F1), 250(F2), 575(F3), 900 (F4) kg ha^{-1} and diammonium phosphate (DAP500 kg ha^{-1}) +Calcium ammonium nitrate (CAN300 kg ha^{-1}) (F5) as a positive control. The experiment was carried out in randomized complete block design (RCBD) spilt plot replicated three times, canola green manure as main plot and fertilizer and varieties combination as sub plots. Semi-field experiment (in pots) was conducted at Egerton university farm, five levels of canola green manure (100, 75, 50 and 0 g kg^{-1} soil) and four levels of the compound fertilizer as used in the field experiment were used in a completely randomized design (CRD) with three replicates. The data shows that canola extract did not have a significant (Pr <0.05) effect on bacterial population density (CFU), where chemical treatment (DDD) restricted the growth of *R. solanacearum* under laboratory experiment. Under field and semi-filed experiments, canola green manure and fertilizer treatments had no significant (Pr <0.05) effect on bacterial population density in the soil nor bacterial wilt incidence. *Kenya karibu* variety it did not show any wilting symptoms of bacterial wilt across the sites and semi-field experiment. Canola did not suppress the growth of *R. solanacearum*; further investigation should be done on other brassica family plants.

Keywords: Green manure, Canola extract, Biofumigation, compound fertilizers, Bacterial wilt, enrich immunomodulator.

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I. Introduction

Bacterial wilt also known as brown rot is potato disease and is one of the most destructive diseases of potato, caused by the pathogen called *R. solanacearum*. [1] reported that is the second most damaging potato disease after late blight in tropical and sub-tropical areas. Globally, the disease is estimated to damage over 950 million USD annually and about 1.7 million hectares of potato are affected by bacterial wilt disease in approximately 80 countries [2]. It is a constraint for potato production mostly in Sub-Saharan Africa. Kenya is one of the most affected country in Africa and affecting over 70% of potato farms and causing yield losses of 50 to 100%. The speed of spread is associated with limited farmer's skills on disease management and prevention, and unavailability of enough healthy potato seeds [3].

The bacteria appear in three races and five biovar, race 3, biovar 2 being important for solanacea family including potato [4], [5]. The bacteria are soil borne, that can spread through infested potato seeds, infested running water, farm equipment, crops residues and infested shoes [32]. The bacteria infest plant from soil through wound that may be caused by nematode, mechanical damage and/ or insect damage on root cortex and colonize the whole plant through xylem vessels, this leads to the obstruction of the vessels and the subsequent development of the typical wilting symptoms due to impaired water conductance [7]. The bacteria can persist in soil for a long period up to five years [8] and the population increases easily with the presence of host plants [9]. Wilting, yellowing, and stunted growth are