EVALUATE THE EFFECT OF ARBUSCULAR MYCORRHIZAL FUNGI AND RHIZOBACTERIA INOCULATION ON PERFORMANCE OF POTATO (*Solanum tuberosum*)

By

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DECLARATION

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

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DEDICATION

This work is dedicated to my mother, Elizabeth and father Aguk for their dedication, love and care, and to my cousin Patrick for being a wonderful guardian. Most of all, I dedicate it to my husband, George and our sons, Baraka and Amani whose understanding, patience and encouragement enabled me to carry out this work smoothly to its conclusion. To God be the Glory for His faithfulness upon my life.

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ABSTRACT

Production of potatoes in Kenya is threatened by diseases, lack of quality potato seeds and low soil fertility. The objective of the current study was to evaluate the contribution of arbuscular mycorrhizal fungi and phosphate solubilizing rhizobacteria inoculants on production of selected Irish potato cultivars

Three greenhouse experiments were established and a follow-up laboratory study conducted. A pot experiment was established using three potato varieties using soil-coco peat based media inoculated with *Pseudomonas spp* and three strains of arbuscular mycorrhizal fungi (AMF). Bacterial wilt experiment was conducted using two potato cultivars inoculated with three strains of AMF and rhizobacteria, while a second experiment was inoculated with three rhizobacteria isolates in single, duo and triple inoculation, three organic amendments and unfertilized control while potato variety Asante grown under asceptic aeroponic conditions was inoculated with three AMF strains to assess their efficacy. The experiments were laid out in complete randomized block design. Performance was based on tuber number, weights, mineral contents, percent mycorrhizal colonization and disease severity.

From the AMF and *Pseudomonas* experiment, the large sized (>5g) tubers was triple in *G. mosseae, G. etunicatum* and *G. intradices* + Pseudomonas while *G. intradices* and *G. mosseae* had the highest number of small sized (<2.5g) tubers compared to unfertilized control. *G. intradices* + *Pseudomonas* in Tigoni had 1.5 times P content than fertilized control. As for root colonization, Tigoni inoculated with *Glomus etunicatum* and *Pseudomonas* had the highest percentage (29%), while no mycorrhizal colonization was observed in all the three uninoculated varieties. Control of Bacterial wilt with AMF and rhizobacteria inoculants was successful in duo inoculates of *G. intradice* and *Pseudomonas, G. etunicatum* and *Bacillus* in clone 387164.4 and *G. intradice* and *Bacillus* for both

cultivars with 0% disease severity and tested negative for latent infection. The disease was more pronounced in Tigoni than in clone 387164.4. *G. etunicatum+Bacillus* had 40% more tuber weight than uninoculated and fertilized control. However, there was no variety treatment interaction on tuber number and mineral tuber content. The results from rhizobacteria experiment in control of bacterial wilt indicated disease suppression in all rhizobacteria inoculated treatment and poultry manure mainly in triple inoculation of *Pseudomonas* + *Bacillus* + *Azoctobacter* that also had the highest tuber weight and tested negative for latent infection in clone 387164.4. There was no effect of the microbial inoculants and organic amendments on tuber grades and on tuber phosphorus content. *Glomus intradices* had the highest percentage of root colonization of 63% compared to 43% in *G. etunicatum* while control was uncolonized in aeroponics experiment. *G. intradices* gave a 22% increase in tuber weight above the control. *G. intradices*, *G. mosseae*, *G. etunicatum* and control had 93, 84, 82 and 73 tubers per plant respectively

Importance of AMF and rhizobacteria as biocontrols and biofertilizers especially when used in combination was demonstrated where, rhizobacteria enhanced nutrient uptake especially P through solubilzation and mineralization while mycorrhizal hypae increased the surface area for nutrient absorption thereby improving plant growth and yield. These beneficial microorganisms are therefore critical for sustainable agriculture as they may reduce the use of fertilizers, pesticides and other chemicals. There is, however, need to test these microorganisms in the field to determine their efficiency and effectiveness.

CHAPTER ONE

1.0 Background information

The potato (*Solanum tuberosum)* belongs to the Solanaceae family and is a tuberous crop which is starchy in nature. It has become the second most important food crop in Kenya after maize and plays a major role in national food and nutritional security and reduction of hunger (Muthoni et al., 2010; KARI, 2005; MoA, 2007). The crop also generates income for a lot of people who include farmers' laborers, traders and processors, and hence contributing to the country's effort of attaining millennium development (MDG) goal 1 of alleviating hunger and reducing poverty. According to the Agricultural Sector Development Strategy (ASDS) 2010-2020, the government's overall vision is to be food secure and be a prosperous nation (Gok, 2010). Approximately 500,000 Kenyan smallholder potato farmers produce about one million tonnes from 120,000 ha of cultivated land area. Kenya's production constitutes 0.3% of the world's total and 6.5% of Africa's production (Muthoni et al., 2010; KARI, 2005; MoA 2005).

The potato has high nutritive value and can supply considerable amounts of energy, minerals and vitamins (Woolfe, 1987; Abong' et al., 2009). The tubers consist of 80 percent water on average and the remaining 20% is the solid matter. It has high quality protein and provides high amount of starch. There are also some of the important vitamins that are found in the potatoes which include thiamine, riboflavin niacin and the vitamin C (Burton, 1989). The minerals that are present include sodium, iron, calcium, sulfur, potassium, phosphorous and magnesium (Woolfe, 1987).

Potato research in Kenya has focused on development and dissemination of high yielding and disease resistant varieties (MOA, 2006). Despite the efforts directed at improving potato production over the years, low productivity remains a major challenge in the sub-sector – the average national farm level yields of 7.3 metric tonnes per hectare (mt/ha) compares unfavorably with on-station research yields of 25 – 35 mt/ha and a potential of 14.5-20 mt/ha under farm level conditions (KARI, 2005). This is

mainly due to increased soil degradation and infertility attributed to intensive cultivation of small farm size owned by farmers, on average, 2 hectares of land in most of the rural farms. Technological advances generated through research to curb this situation have not translated to increased efficiency and resource productive capacity.

The major constraint to potato production in the cool highlands of Kenya is the rapid decline in soil fertility occasioned by continuous cultivation without adequate replenishment of mined nutrients (Kiiya et al., 2006; Nganga et al., 2008). Potato is a heavy feeder on soil nutrients (Sikka, 1982, Clark et al., 1998) mainly nitrogen, phosphorous and potassium which must be replenished to sustain the soil fertility status and to maintain or improve the crop yields. The amount of nutrients uptake by a potato crop is closely related to yield; usually twice the yield will result in twice the removal of nutrients. Potatoes also have a relatively shallow root system with most roots located in the top 2 feet of soil and cannot therefore access the nutrients deep within the soil. The potato plant requires adequate supplies of several essential elements for proper growth and function.

At present, use of biological approaches is becoming more popular as an additive to chemical fertilizers for improving crop yield in an integrated plant nutrient management system. In this era where sustainable crop production is becoming necessary, the plant–microbe interactions in the rhizosphere plays a pivotal role in transformation, mobilization, solubilization of nutrients from a limited nutrient pool, and subsequently uptake of essential nutrients by plants to realize their full genetic potential and enhance plant productivity (Burd et al., 2000; Cocking, 2003). Mutually beneficial interactions between plants and microbes are frequent in nature.

Mycorrhizal fungi are soil-dwelling, root-inhabiting fungi that colonize the fine absorbing roots of more than 95% of land plants. Mycorrhizae are an integral part of most plants in nature (Giazninazzi et al., 1982) and were found to occur in 83% of dicotyledonous and 79% of monocotyledonous plants investigated by Wilcox (1996). In AMF-plant symbioses, the plant supplies AMF with C in the form

of photosynthates; about 5 to 85% of C depending on the plant species and its dependence on the association (Treseder and Allen, 2000). In return, the plant gain from the use of the mycelium's extremely large surface area to absorb water and inorganic mineral nutrients from the soil. Many of these minerals are in forms unavailable to a non-colonized plant hence AMF are greatly beneficial to plants growing in nutrient-poor soils and reduces the need for heavy fertilization (Barea and Jeffries, 1995). By virtue of their roles in soil aggregate formation and stabilization, they increase C and N storage (Zhu and Miller, 2003; Rillig, 2004a), thereby reducing greenhouse gas emissions. It is also reported that many mycorrhizal fungi secrete antibiotics, protecting roots from pathogenic fungi such as *Phytophthora* and *Armillaria*, nematodes (small invasive worms), and bacteria thus reducing need for fungicides. Furthermore, in areas polluted by toxic heavy metals, fungi can buffer their plant partners against harm. For instance incase of excess manganese (Mn) in soil toxic to crops, arbuscular Mycorrhiza fungi may alleviate the toxic effects by producing compunds that affect the balance between Mn-reducing and Mn-oxidizing microorganisms in the mycorrhizosphere and thus affect the level of extractable Mn in the soil (Nogueira, et al., 2007).

In soil with low P bioavailability, free-living phosphate-solubilizing bacteria may release phosphate ions from sparingly soluble inorganic and organic P compounds in soil (Kucey et al., 1989), and thereby increase soil phosphate pool availablability for the extraradical AM fungal hyphae to pass on to the plant (Smith and Read, 1997). Management of such interactions is a promising approach for low-input agricultural technologies (Bethlenfalvay & Linderman 1992; Gianinazzi & Schüepp 1994; Jeffries & Barea 2001). Several studies have demonstrated synergistic interactions between phosphate-solubilizing bacteria and AM fungi (Barea et al., 1997; Kim et al., 1998). This project proposes to study the effects of arbuscular mycorrhizal and rhizobacteria inoculants on production and nutritional properties of selected Kenyan potato cultivars.

1.2 Problem statement and justification

While the average potato yields in North America and Western Europe often reach 40 tonnes per hectare, yields in developing countries such as Kenya are usually below 20 tonnes per hectare (FAO, 2008). Kenyan production of 980,000 t from 120,000 ha, indicate national average yields of about 7- 8 t/ha. This is low compared to the 25t/ ha that can be attained by progressive farmers under rain-fed conditions (Kinyae et al., 2004; Low, 2000; Aliguma, 2002). This yield gap can be attributed to high incidences of diseases, particularly late blight and bacterial wilt, the use of low quality seed potatoes degenerated by viruses, poor general crop husbandry and most importantly inadequate soil fertility management (MoA 2005; Nganga et al., 2002). Low soil fertility is a widespread problem that requires urgent attention for high productivity to be realized within a short production period, especially in the wake of changing climate.

Potato being second to maize in importance requires that the country up scale production of the crop through better and improved agronomic practices. The need for low-cost and sustainable technical solutions to solve the soil fertility problem of smallholder farmers is therefore apparent. Smallholder farmers have few technological options which are compatible with both the physical and chemical status of their soils and their poor socio-economic conditions. Smallholder farmers' access to external inputs such as improved seed, fertilizers and pesticides has declined (Odame, 1997). Therefore, use of beneficial micro-organisms such as arbuscular mycorrhizal fungi (AMF) is an attractive technological option in improving plant productivity (Brundrett and Jupiter, 1995).

Potato production in Kenya is mainly rain-fed. This has resulted in low yields which correspond to periods of drought in the country. Increase in production has mainly been due to increase in areas under potato cultivation with severe environmental degradation since this is done at the cost of clearing natural forests, savannas and grasslands. Kenya's 1994-96 Development Plan recognized the fact that Kenya's agricultural production can hardly be increased by expanding the area under

cultivation. With increased global population pressure, land is becoming increasingly limited. The world's demand for food will have to be met by intensifying agriculture on land already in cultivation. Breeding programmes to determine tolerance of potatoes have been established, but acceptable cultivars with good tolerance to bacterial wilt are yet to be identified in Kenya (Ateka et al., 2001).

Due to intense cultivation without adequate replenishment of mined nutrients, there has been rapid decline in soil fertility (Kiiya et al., 2006; Nganga et al., 2008). If the soil fertility is not good, the crops are not in optimal condition, and are thus more susceptible to diseases and pests further lowering productivity levels, threatening the livelihoods of rural communities. Use of fertilizers is indispensible though it is done at an extra cost and expected production is not realized in most farms due to poor or degraded soils (Baligar and Bennett, 1986). This is especially so with phosphate fertilizers which when applied to the soil are rapidly converted into insoluble complexes hence low plant utilization (Vassilev and Vassileva, 2003). This leads to the need of frequent application of phosphate fertilizers, but its use on a regular basis has become a costly affair and also environmentally undesirable (Reddy et al., 2002).

There is therefore need to improve food production without necessarily using high dosages of farm inputs. Soil microorganisms can be used to decrease the use of fertilizers, pesticides and other chemicals. Among soil microorganisms, arbuscular mycorrhizal (AM) fungi and *Rhizobium* spp. are known to promote plant growth and protect against pathogens (Grosch et al., 2005). Phosphate solubilizing microorganisms (PSMs) convert insoluble phosphates into soluble forms through the process of acidification, chelation, exchange reactions and production of gluconic acid (Rodriguez et al., 2004; Chung et al., 2005). Integration of arbuscular mycorrhizal fungus during potato farming, especially on established varieties, is hence a promising approach for sustainable production especially in Kenyan context (Akhtar et al., 2008).

1.3 Objectives

1.3.1 Overall objective

To quantify the contribution of arbuscular mycorrhizal fungi and phosphate solubilizing rhizo-bacteria inoculants on performance of selected potato cultivars.

1.3.2 Specific objectives

1. Evaluating effectiveness of Arbuscular Mycorrhizal Fungi (AMF) and rhizobacteria inoculants on performance of selected potato cultivars.
2. Assess performance of elite AMF, rhizobacteria strains and organic amendments on suppression of bacterial wilt.
3. Evaluate effect of commercial AMF strains on yield of minitubers from potato grown in aeroponics system.

1.3.3 Hypotheses

1. Co-inoculation of AMF and rhizobacteria will enhance potato peformance.

2. Co- inoculation of AMF and rhizobacteria inoculation have enhanced effect in control of bacterial wilt.

3. AMF inoculation will enhance yield of potato in aeroponic system.

CHAPTER TWO

LITERATURE REVIEW

2.1 Potato production and utilization in Kenya

The potato (*Solanum tuberosum*) is widely grown in the world and ranks fourth in production, after wheat, maize and rice (Qu et. al., 2005). Among the tuber and root crops, Irish potato is the most utilized followed by cassava, sweet potato and yams. Today, the market is expanding rapidly as potatoes are increasingly becoming popular as a source of affordable food for growing urban populations with a preference for potatoes in processed forms such as fries and crisps providing a growing domestic market that presents a valuable opportunity for smallholder farmers (Walingo et al., 1997, Abong' et al., 2010).

The European settler farmers introduced potatoes in Kenya initially in Kiambu, Murang'a and Nyeri districts in the late 19th century primarily for domestic consumption and later, for export. Overtime, the Irish potato has grown to become the second most important food crop after maize in terms of production and utilization in Kenya (MoA, 2008; CIP, 2006) and plays a major role of ensuring food security for the ever increasing population and provide a source of income for the local people (FAO, 2010). It is cultivated both as a subsistence and commercial crop. This twin role of potato assist farmers come out of subsistence farming and poverty since it has reduced risk exposure (FAO 2010). It is mainly cultivated by small-scale farmers in the high altitude areas between 1,500 and 3,000 meters above sea level (MoA, 2005). These areas include; the slopes of Mt. Kenya (Meru districts, Embu, Kirinyaga, parts of Laikipia, Nyeri); both sides of the Aberdare range covering parts of Nyeri, Murang'a, Maragua, Thika, Kiambu and Nyandarua districts; the Mau Escarpment (Narok, Nakuru, Bomet, Uasin Gishu, Koibatek) districts; South and North Nandi districts; Trans Nzoia, Mt Elgon, Keiyo and Marakwet. Small acreages are cultivated in Kericho and Kisii highlands and isolated patches in the Taita hills of Taita Taveta districts (Kirumba., et. al., 2004). Potato in Kenya is grown

by about 500,000 farmers, cultivating 120,000 hectares per season with an annual production of about one million tonnes in two growing seasons (MoA, 2008). Its importance is attributed to its high nutritive value, good productivity and good processing qualities for starch, flour, bread, soap, alcohol, weaning foods and animal feed.

Currently over one billion people worldwide are undernourished due to insufficient food consumption (FAO, 2009). Ensuring adequate quantity of potato will enhance food security and reduce poverty. However, potato yields in the country are generally low (Kaguongo et al., 2008; Muthoni et al., 2010). On-farm yields achieved by smallholders are much lower than 10 t/ha compared to those under optimal conditions in research stations of over 40 t/ha (Lung'aho et. al., 1997). Low production is mainly attributed to low soil fertility, occasioned by continuous cultivation without adequate replenishment of mined nutrients (Kiiya et. al., 2006; Kaguongo et. al., 2008). The most limiting soil nutrients to potato production in Kenya are nitrogen and phosphorus (Recke, 1997). This is because fertilizers are usually applied below the recommended rate (90 kg N/ha + 230 kg P_2O_5/ha) (Kaguongo et. al., 2008).

The application of organic matter to the fields is limited because of the many alternative uses in the farm, mainly as fodder. Cattle manure when used is usually of low quantity and quality far beyond the recommended rates (Muthoni et. al., 2010). This is worsened by inadequate crop rotation due to small farm sizes (Kaguongo et. al., 2008) and the fact that most production is mainly rain-fed. Diseases such as potato blight (*Phytophthora infestans*), bacterial wilt (*Ralstonia solanacearum*) and viruses are recognized as primary constraints to production (Lemaga et. al., 1997; Mienie, 1997; Barton et al., 1997). In the absence of continuous sources of good quality seed, many growers opt to use their own farm saved seed or those obtained from markets. Such practices contribute towards sub-optimal yields as well as spread of diseases such as bacterial wilt (*Ralstonia solanacearum*), a serious disease of potatoes, and viruses (Kaguongo et. al., 2008).

2.2 Bacterial wilt

Cultivation of potato is promoted for its high development potential in Asia and Africa and as a possible decisive weapon in the fight against starvation. Unfortunately, potato is susceptible to numerous pathogens for which control methods are hard to come by since there is shortage of disease free seed-tuber (Qu et. al., 2005) resistant or tolerant cultivars are lacking and certified seed is expensive or unavailable. (Michieka, 1993; Kinyae et. al., 1994; Barton et. al., 1997; Kinyua et. al., 2001). Potatoes are usually cultivated from earlier generation seed potatoes, this process of vegetative propagation can cause build up of diseases and eventual spread from one region to another. In Kenya bacterial wilt was first reported in 1940s and has since spread to most potato growing region (Michieka, 1993).

Bacterial wilt (brown rot of potatoes) is caused by race 3 biovar 2 of *Ralstonia solanacearum*. It ranks as second most important potato disease after late blight (Momol et. al., 2000; Felix et. al., 2010; Lemaga et. al., 2001). The bacterium invades the root of the host plant and aggressively colonizes the xylem vessels causing wilting (Tahat et. al., 2008a; Smith et. al., 1995). Disease control has been difficult because of the localization of the pathogen inside the xylem and its ability for survival at depth in the soil. In addition, there is no chemical control of the bacterial wilt disease (Hartman et. al., 1993). Bacterial wilt is difficult to control due to high variability of the pathogen (Sequeira, 1992), it can also survive in the soil for long period of time in absence of the host plants (Jones, 1997; Tahat et. al., 2008b). The incidence of the disease is exacerbated under conditions of moisture stress, low soil fertility and low pH (Woltz and Jones, 1968; Shekhawat et. al, 1978) since low pH and organic matter contents provide a conducive environment that support multiplication of the pathogen (Ramesh and Bandyopadhyay, 1993). The combined effect of soil physical limitation; high bulk density, low water conductivity and reduced effective rooting depth of crops on the upper parts of terraces have been

shown to greatly affect crop performance (Siriri, 1998) and nutrient depletion results in reduced crop productivity leading to declining plant resistance to the pathogen infestation (Muchovej et. al, 1980). It is reported that bacterial wilt is more widely spread on heavy than lighter soils (Shekhawat et. al 1978) and that the presence of the bacterium varies with soil depth (Graham and Lloyd, 1979) and with soil moisture and temperature (Shekhawat and Perombelon, 1991)

Quarantine measures necessary to avoid spread of the disease, often restrict the production of seed potatoes and limit the commercialization of ware potato (Martin et. al., 1985). Besides the available cultural practices methods like crop rotation, use of clean seeds, planting in non-infested soils and use of tolerant varieties may provide limited control since all the control measures have individual practical, technological and economic limitation (Jinnah et. al., 2002, Khalequzzaman et. al., 2002, Muthoni and Nyamongo, 2009). For instance, crop rotation using resistant varieties was considered as an effective alternative, but the large number of hosts has reduced its effectiveness (Ji et. al., 2005). The use of antagonistic microorganisms is therefore one of the key strategies to control soil borne diseases like bacterial wilt (Raupach and Kloepper, 1998; Pal and Garedener, 2006).

2.3 Arbuscular Mycorrhizal Fungi (AMF)

Vesicular arbuscular mycorrhizal (VAM) fungi (Zygomycetous fungi from the order Glomales) form the most widespread symbiosis of the plant kingdom and colonize more than 80% of vascular plants **(**Barek et. al., 2001**)**. Symbiosis refers to an association of living organisms that benefits both partners, enabling them to survive, grow and reproduce more effectively and above all, acquire increased resistance to environmental stresses such as drought, cold and root pathogens **(**Sylvia and Williams, 1992; Mohan, 2000**).** They are therefore considered essential for ecosystem functioning (Koide and Mosse, 2004; Van der Heijden, 2002) because they play a fundamental role in soil fertility and in the maintenance of stability and biodiversity within plant communities (Giovannetti and Avio, 2002). The

classification of AMF is based on the structure of their soil-borne resting spore, biochemical properties and molecular studies (Morton and Benny, 1990). The highest number of mycorrhizal spores and root infestation was observed in potato (Sharif et. al., 2006). The AMF spores germinate and thick-walled hyphae penetrate the host root causing internal infection as the hyphae and develop an extramatrical mycelium in the root cortex without damaging the integrity of the cells (Strack et. al., 2003). The most prominent results of root mycorrhizal colonization are, among others, an improved nutrient status of the host plant (Smith and Read, 1997) and a bio-protective effect of mycorrhization against soil borne fungal pathogens (Singh et. al., 2000; Azcon-Aguilar et. al., 2002; Xavier and Boyetchko, 2004; St-Arnaud and Elsen, 2005; St-Arnaud and Vujanovic, 2007). Mycorrhizal fungi themselves may release exudates that selectively influence the microorganisms in the rhizosphere (Rillig et. al., 2002). Mycorrhizal colonization has also been shown to indirectly affect the microbial community in the rhizosphere by its effects on root morphology (Berta et. al. 1990, 1993; Hetrick 1991), rhizosphere pH (Bago and Azcon-Aguillar, 1997), nutrient content (Li et. al., 1991) and enzyme activity (Tarafdar and Marschner, 1994) as well as on soil structure (Tisdall, 1991; Neergaard-Bearden and Petersen, 2000; Rillig et. al., 2002).

Mycorrhizal fungi contribute significantly to the phosphorus nutrition of plants, particularly under low phosphorus conditions (Barea et. al., 2008). Under drought conditions the uptake of highly mobile nutrients such as NO^{3-} can also be enhanced by mycorrhizal associations (Ázcón et. al., 1996; Subramanian and Charest, 1999). It is therefore evident that mycorrhizal association results in stronger plants enabling them to withstand both biotic and abiotic stress. However, agronomic practices can certainly alter characteristics of root colonization (particularly reducing arbuscule development) and markedly decrease AM fungal biomass per plant, including both biomass in roots and in soil (Smith and Read, 2008; Balzergue et. al., 2011) thereby lowering the inoculum in the soil and subsequent colonization such as a result of frequent use of P fertilizer, long fallow periods, cultivation of non-host

crops (especially members of Brassicaceae), or frequent soil tillage that disrupts networks of AM fungal hyphae in soil (Jansa et. al., 2006, Oehl et. al., 2003; 2005).

The use of fungicides is generalized in modern agriculture for the control of fungal diseases. Fungicide treatment have however been known to reduce or eliminate AM activity (Sukarno et. al., 1993) by inhibiting spore germination and hyphal length of the arbuscular mycorrhizal fungus (Chiocchio et. al. 2000). The effects may be rate-dependent (van Faassen, 1974). The interaction between AM systems and agrochemicals in the soil is an important consideration in the development of appropriate management technologies of AM fungal populations (Nelson and Khan, 1992). The bio-protective effect against soil borne fungal pathogens seems to depend on several biotic factors such as the host genotype, the AMF isolate and the degree of mycorrhization. Plant species can have preferences for individual AM fungi, resulting in different densities of colonization (Smith et. al., 2009). Therefore plant responses to AM colonization vary from highly positive to negative (Johnson et. al., 1997; Smith and Smith, 2011).

2.4 Rhizobacteria

Phosphorus is one of the key macronutrient required for plant growth and metabolism. It plays an important role in energy transfer through the formation of energy-rich phosphate esters and is also an essential component of macromolecules such as nucleotides, phospholipids and sugar phosphates (Marschner et. al., 1995). Much of the inorganic phosphate applied to soil as a fertilizer is rapidly converted to unavailable forms of aluminum, calcium, or iron phosphates with low solubility hence unavailable to plants (Yadav and Dadarwal, 1997; Wakelin et. al., 2004). As such, the concentration of soluble phosphorus in soil is usually very low, normally at levels of 10 μM or less and has very low mobility (Schachtman et. al., 1998). In addition, unfavourable pH and high reactivity of aluminium

and iron in soils decrease phosphorus availability as well as phosphorus fertilizer efficiency (Börling et. al., 2001; Hao et. al., 2002). Microorganisms are central to the soil phosphorus cycle and play a significant role in mediating the transfer of phosphorus between different inorganic and organic soil fractions, subsequently releasing available phosphorus for plant acquisition through solubilization with aid of organic acids they synthesize (Deubel and Merbach, 2005).

Given the limited access of most farmers to phosphate fertilizers due to their high cost, it is necessary to identify and incorporate into cropping systems efficient strains of rhizobacteria that can mobilize unavailable phosphorus from soil phosphate pools into forms that can be readily assimilated by plants. *Pseudomonas, Bacillus*, *Enterobacter*, *Azospirillum* and *Rhizobium* are considered as the most powerful solubilizers (Whitelaw 2000). Dual inoculation involving mycorrhizae and *Azotobacter*, Rhizobium and rhizobacteria performed better than single inoculations of mycorrhizae (Pavan et. al., 2011).

2.5 Biological control of bacterial wilt using Arbuscular mycorrhizal Fungi (AMF) and rhizobacteria

Microbial pathogens are annually responsible for the loss of 25% of worldwide production (Priou et. al., 1999; FAO, 2008). Development of effective and sustainable techniques for the management of soil-borne plant-pathogens is a subject of increasing interest (Ji et. al., 2005). Despite its high potential, potato is susceptible to many pathogens. Its genetic complexity combined with its small number of resistance genes limits genetic control methods to a few viral diseases (Priou et, al., 1999; Gebhardt and Valkonen, 2001). Consequently, up to this point the potato's prosperity relies mainly on sanitary and phytosanitary measures, and the development of microbiological control is a worthy and challenging alternative (Latour et. al., 2008). Potato relies on the development and use of

environmentally risk-free strains, which are able to colonize the potato plant and express antagonism under every cultivation condition cutting down the need of inputs, including pesticides and fertilizers. Bio-control methods represents a significant complement to other control methods, which are based on prophylactic measures, chemical treatments or genetic approaches. This is even more important considering that many diseases affect underground organs, which are usually out of reach of germicidal treatments. A variety of soil microorganisms have demonstrated antagonistic activity in the control of various soil-borne plant pathogens, including bacterial wilt pathogen. Several researchers have observed improved disease control using various bio-control organisms such as *Trichoderma* sp. (Roberti et. al., 1996; Lewis et. al., 1998; Adekunle et. al., 2001). Plant Growth promoting rhizobacteria (PGPR) influence the symbiotic association between plant and other microorganisms including AMF. The most common rhizobacteria are *Pseudomonads*, *Azotobacter* and *Bacillus* (Vessey et. al., 2003). They reduce pathogenic attacks by producing antibiotics (Whipps et. al., 2001). There are studies on a related *Solanaceae* (tomato) that demonstrated mycorrhiza's ability to limit the density of the soft-rot pathogen *Pectobacterium carotovorum* in the rhizosphere, to induce a systemic response to *Phytophtora* infection (Garcia-Garrido and Ocampo, 1988; Pozo et. al., 2002) and Glomus mosseae suppression of *R. solanacearum* (Tahat et. al., 2009). Mycorrhizal fungi are among the most frequent rhizosphere microorganisms, and they can also influence the growth and health of plants (Buée et. al., 2009), but their interactions have however been long been underestimated. AMF is bio-trophic in nature, surviving within the root system until crop maturity, and hence may give mechanical strength to plant roots against soil-borne plant pathogens (Sharma et. al., 1992) by inducing plant defense proteins i.e. PR proteins (Agrawal et. al., 2002; Bart et. al., 2002; Van Loon et. al., 2006), increasing competition for infection sites (Vigo et. al., 2000), conserve the root system by compensating for the loss of root functional and biomass caused by soil borne pathogens (Cordier et. al., 1996), and increasing nutrient uptake resulting in more vigorous plants and hence increasing

resistance or tolerance to pathogen attack (Linderman, 1994). It also increases phosphorus uptake when plant is grown in nutrient poor soils (Smith and Read, 2008). Therefore, the potential bio-control effect of mycorrhizal fungi on *Solanaceae* diseases needs to be further developed.

2.6 Methods of potato seed production

Seed tubers are often the main source of diseases and pest which reduces yield considerably. Farmers usually use conventional method using tubers from individual plants that appear to be visually free of diseases. A number of disease-causing pathogens like bacterial wilt can remain latent within seed and when eventually planted result to disease build up which severely reduces yield (Badoni and Chauhan, 2010). Therefore, convenient methods of production is important to ensure quality clean seeds.

Certified seed tubers is mostly based on in-vitro plantlets from meristem tissue culture which are then multiplied and acclimatized by hardened-off (KARI, 2007). They are then planted at high densities in greenhouse in beds or pots containing sterilized sand or in hydroponics culture to produce pre-basic minitubers which have to be multiplied for three generations in the field to produce basic seeds. In modern horticulture, different soil-less production techniques such as aeroponics (Otazu, 2010) have been developed. This method involves suspending the root system in a dark well-aerated chamber and supplying a solution of water and mineral nutrients with a mist device. Due to sequential harvesting and adequate space for roots and tuber development, this system has rapid seed multiplication resulting to higher yields compared to other techniques (Otazu, 2010). This indicates the effectiveness of aeroponics in seed production. However, the system requires highly trained specialists, uninterrupted power supply and high cost of maintenance which limit the number of farmers to be involved in potato-seed production using aeroponic system (Chiipanthenga et. al., 2011). However, most farmers will have access to quality certified seeds (CIP, 2008) which will enhance production.

Outline of the thesis

This thesis is composed of seven chapters. Chapter three evaluates the effectiveness of AMF and *Pseudomonas* inoculants affect the performance of potato in soil-cocopeat based media. Three potato cultivars commonly grown by farmers were examined using three commercial AMF strains and *Pseudomonas* under different microbial inoculants of single and duo inoculants. One month soil solarization was considered as an effective means of destroying harmful pathogens and pests without affecting beneficial soil microorganisms.

In chapter four and five, the effect of AMF and rhizobacteria inoculant as biocontrols for bacterial wilt of potato was examined. The objective was to assess the performance AMF and rhizobacteria on suppression of bacterial wilt. Two cultivars were used in both cases, Tigoni and Clone 387164.4 which were chosen to represent highly susceptible and less susceptible cultivars respectively. In chapter four, three commercial AMF and rhizobacteria were studied in various combinations. In chapter five, three rhizobacteria isolates were studied in single, duo and triple inoculation and further considered organic amemdments of poultry, compost and cow manure. In Chapter 6, the objective was to evaluate commercial AMF on yield of potato minituber in aeroponic system determine their effectiveness in enhancing potato minituber yield. Chapter 7 provides a general conclusion of the results described in the previous chapters and recommendations.

CHAPTER THREE

Evaluation of Arbuscular Mycorrhizal Fungi (AMF) and Rhizobacteria inoculants on performance of potato (*Solanum tuberosum)*

3.1 Abstract

The study was designed to evaluate the effect of three commercial Arbuscular Mycorrhizal Fungi AMF; *G. mosseae*, *G. etunicatum* and *G. intradices* and rhizobacteria (*Pseudomonas*) singly and in combination on three Kenyan potato genotypes (*Solanum tuberosum)* namely; Tigoni, Kenya mpya and Asante. Potatoes were planted in a completely randomized block design with four replicates. Data was subjected to analysis of Variance to investigate the effect of AMF and *Pseudomonas* on minituber weight and number, shoot weight, shoot Phosporus and root mycorrhizal colonization on potato.

The large sized (>5g) tubers was three times in *G. mosseae, G. etunicatum* and *G. intradices* + *Pseudomonas* compared to control with no fertilizer, while *G. intradices* and *G. mosseae* had the highest number of small sized (<2.5g) tubers. There was no effect of the microbial inoculants and variety on the number of medium tubers. All inoculated treatments and fertilized control had double tuber weight compared to that of unfertilized control. Tigoni inoculated with *G. intradices* + *Pseudomonas* had 1.5 times higher P content than fertilized control. The number of indigenous spores was higher in treatments that had no mycorrhizal inoculation, while dual inoculation with *G. mosseae* and *Pseudomonas* had the highest inoculated spore count. As for the root mycorrhizal colonization, Tigoni inoculated with *Glomus etunicatum* and *Pseudomonas* had the highest percentage (29%), while no mycorrhizal colonization was observed in unfertilized control. AMF and rhizobacteria dual inoculant significantly increased tuber yield and shoot weight of potatoes grown in pots. These promising results indicate the importance of microorganisms in crop production. However; there is need for extensive field trials of these performances of bio-inoculants under varied soil and farm environment.

Key words: Potato, *Glomus intradices*, *Pseudomonas spp*, root colonization

3.2 Introduction

Potato is an important food crop in Kenya constituting 0.3% of the world's total and 6.5% of Africa's production since it plays a major role in national food and nutritional security, reduction of hunger, generation of income and employment (KARI, 2005; Muthoni and Nyamongo, 2010; MoA, 2007; Olanya et. al., 2006). Potato production has increased due to economic decline of competing crops such as maize and pyrethrum, and increasing demand from consumers and processors (Kabira, 2002). Kenya's production constitutes. The major constraint to potato production in Kenya is low soil fertility (Kiiya et. al., 2006; Nganga et. al, 2008), high incidences of pests and diseases, particularly late blight and bacterial wilt, the use of low quality seed potatoes degenerated by viruses, (MoA 2005; Nganga et. al., 2002), and the high demand of available soil nutrients (Struik et. al., 2006). Potato plants are relatively inefficient at acquiring soil Phosphorus (P) (Pursglove and Sanders, 1981), a condition further aggrevated by the low P solubility forms of phosphates of Fe, Al and Ca (Schachtman et. al., 1998). Despite the high abundance of P in parent rock and application as fertilizer its availability is still limited to plants due to rapid P soil fixation (Dey, 1988).

An alternate plant strategy for coping with P deficiency is the use of beneficial microorganisms as biofertilizers and reduced use of fertilizer inputs. The arbuscular mycorrhizal fungi and rhizobacteria have the potential to alleviate P constraints and enhance crop productivity, soil and plant nutrition (Lambers et. al., 2009, Smith et. al., 2011).

Mycorrhizas are highly evolved mutualistic associations between soil fungi and plant roots (van der Heijden et. al., 2006) of over 80% of terrestrial plants (Rinaudo et. al., 2010) and mediate soil nutrients uptake especially P and water through fine hyphae that penetrate narrower soil crevices and increase the root surface area for absorption (Goodwin 1992) as well as increase root longevity (Eissenstat et. al., 2000). Potato has a low root density (Muchovej, 2002; Pursglove and Sanders, 1981) and high growth potential, and arbuscular mycorrhizal symbiosis may be of particular significance in coping with P deficiency. The fungi are biotrophic (Smith and Read, 2008 and Sanders and Croll, 2010) and

may release exudates that selectively influence the microorganisms in the rhizosphere (Rillig et. al., 2002). Significant yield increase due to AMF has been recorded in production of potato minitubers (Duffy et. al., 2000; Gabriela et. al., 2009). Rhizobacteria are aggressive root colonizers that enhance nutrient availability especially P through mineralization and solubilization of organic and inorganic P by secretion of phosphatase enzyme and organic acid into orthophosphate forms that can be assimilated by plants (Idris et. al., 2009). They also produce siderophores (chelators) that enhances micronutrient availability like Fe by reducing Fe^{3+} to Fe^{2+} that is readily utilized by plants (Glick, 1995). Co-inoculation of rhizobacteria and AMF have greater effect on plant performance than individual inoculants since rhizobacteria can stimulate mycorrhizal root colonization (Toro et. al. 1997).

The aim of this study was to investigate the effects of dual inoculation with AMF and rhizobacteria on performance of micropropagated potatoes (*Solanum tuberosum*) transplanted to a soil-coco peat based substrate in the green-house.

3.3 Materials and Methods

3.3.1 Site Characterization

The study was carried out in a screenhouse at Upper Kabete field station, University of Nairobi in 2012. The field station is located at 1^0 15'N, 36^0 44 'E with an elevation of 1800 metres A.S.L. and categorized under agro-ecological zone III (Sombreak et. al., 1982). The climate is typically sub-humid with mean annual temperature varying from 12^oC- 23^oC and total annual rainfall ranging between 1200-1800mm. Rainfall is bimodal in distribution, with the long rains from March-May and short rains from October-November. Soil used in the experiment was collected from KARI-Tigoni in Limuru located 40 km North-west of Nairobi with average annual rainfall of 1096 mm and mean annual temperature between 12 and 24°C. The area is situated at latitude of 1°15' S and longitude 23°

46' E, the soil was sampled from plots which had been left fallow under grass cover for 15 years and with humic nitisol soil, mainly clay loam. The soil chemical characteristics are shown in table 3.1.

Table 3.1: Chemical characteristic of soil used in the current study

Parameter	Values
pH ($CaCl_2$)	6.0
Available phosphorus (Olsen) (mg/kg)	12.4
Cation exchange capacity (CEC) (cmol/kg)	16.0
Sand (%)	11.8
Silt (%)	54.0
Clay (%)	34.1

3.3.2 Soil preparation and experimental layout

The soil was sieved through 5mm mesh sieve to remove large soil clods and debris then sterilized by solarization by covering the soil with polythene sheet and left exposed to the sun for one month (Ramesh Pokharel, 2011). Sterilized coco-peat was mixed with the soil at the ratio of 1:1 approximately 3kg per pot to enhance water infiltration and holding capacity, and to reduce compaction of soil, which is important for proper tuberization.

Potato varieties; Kenya Mpya, Tigoni and Asante commonly grown by farmers were used in the study. Four week old in-vitro plantlets from Genetic Technology Limited (GTL) were hardened for three weeks in trays containing sterilized sand then transplanted in 18cm diameter plastic pots. Plates were placed at the bottom to collect leachate and reduce cross contamination. Each pot initially contained two kilograms of soil-cocopeat mix and two plantlets. Microbial inoculants were applied directly into the planting holes at the time of planting except for 2 controls, one with fertilizer treatment and the other with no fertilizer treatment. Five microlitre (10^8cfu/ml) of rhizobacteria strain *Pseudomonas fluorescent* isolated from rhizosphere of potato was applied with of each of the three commercial

strains of AMF that were supplied by Dudutech division of Finlays Horticulture in granular form with infected roots. The three strains namely *Glomus intraradices, G. etunicatum,* and *G. mosseae* were each applied at 25g in the planting hole. There treatments were: *Glomus intraradices G. etunicatum, G. mosseae, G.intraradices + Pseudomonas, G. etunicatum + Pseudomonas, G. mosseae + Pseudomonas, Pseudomonas,* control with fertilizer and control without fertilizer on the three potato cultivars. After two months, hilling was done through addition of one kilogram of soil mixed with cocopeat to each of the pots. Every three days 300ml of calcium nitrate based nutrient solution was applied to the plantlets for the entire growth period of 90 days when the potatoes were ready for harvesting except for one of the control where only water was applied (Otazu et. al. 2010). The experiment was randomized complete block design (RCBD) with factorial arrangement, variety and inoculum being factors under consideration and each treatment replicated four times.

3.3.3 Measurement of biomass content and yield.

Plants were harvested at 90 days after planting; tubers were graded, counted, and weighed in grams per plant. Based size on weight, a single tuber was graded as follows; <2.5g for small, 2.5-5g for medium and >5g for large. The roots were cut at the base of the stem and fresh shoot weight per pot recorded, the plant shoots were placed in brown sugar paper bags and oven dried at 70^0C for 48hrs. The dry weight was recorded and the shoots grounded to <0.25mm in a stainless mill for phosphorus determination using microwave-assisted acid extraction/dissolution of plant material (Laboratory test method, Crop Nutrition Laboratory Services, 2013).

3.3.4 Assessment of mycorrhizal root colonization.

Fine roots were washed free of adhering soil and used for analysis of mycorrhizal colonization. They were chopped into fragments, one centimeter long and cleaned using 10% KOH, bleached in ammonium and hydrogen peroxide solution, neutralized in 1% HCL and stained with 0.05% trypan blue in acid glycerol according to method described by Koske and Gemma (2005). The stained roots

fragments were mounted on slides in polyvinyl alcohol-lactic acid-glycerol solution and examined under a microscope at magnification power of X40 to obtain the percentage of roots colonized by mycorrhizal fungi. Percentage root colonization was calculated following formula by Giovannetti and Mosse, (1980):

$$\text{Root colonization (\%)} = \frac{\text{No. of colonized segments}}{\text{Total No. of segments examined}} * 100$$

3.3.5 Estimation of AMF spore population in soil after harvesting.

After harvesting, 50g of thoroughly mixed soil without roots from each pot was weighed and used for spore extraction by combination of wet-sieving and decanting (Gerdeman and Nicolson, 1963), and sucrose-centrifugation techniques (Jenkins, 1964). The spores were examined and counted under a dissecting microscope.

3.3.6 Data management and analysis.

Data was subjected to analysis of variance (ANOVA) using proc GLM least significant difference test for the variables conducted using Statistical Analysis System (SAS version 9). Due to non-homogeneity of variances in dry shoot weight and indigenous spore count, data were log transformed. Pearson correlation analysis was performed to determine the relationship of mycorrhiza and the different parameters. Differences at $P \leq 0.05$ were considered significant.

3.4 Results

3.4.2 Effect of AMF and *Pseudomonas* inoculation on potato grade, yield, biomass and shoot P content

The different potato minituber sizes under different microbial inoculants are presented in Fig. 3.1.

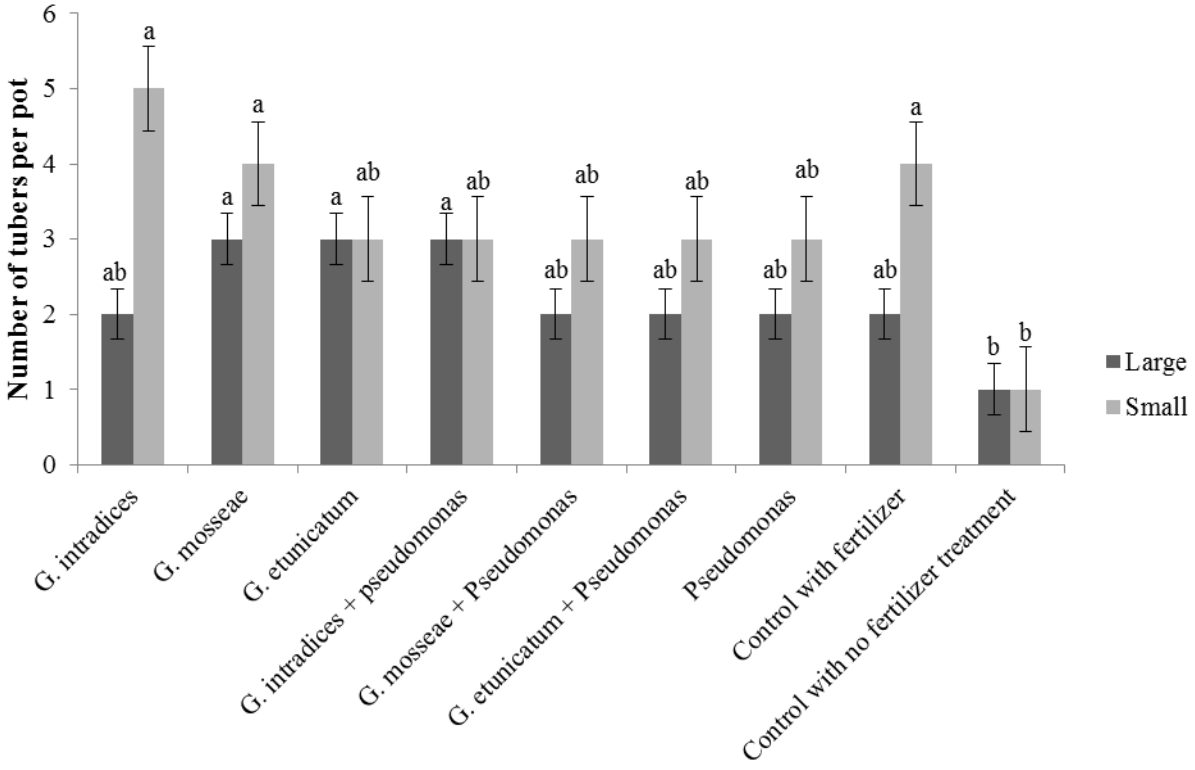


Figure 3.1: Potato grade as influenced by microbial inoculants. Bars indicate standard error bars. Bars with the same letter are not significantly different at P≤0.05

The large sized (>5g) tubers were three times more in *G. mosseae*, *G. etunicatum* and *G. intradices* + *Pseudomonas* compared to unfertilized control. Treatments with *G. intradices* and *G. mosseae* had the highest number of small sized (<2.5g) tubers compared to control with no fertilizer. There was, however, no effect of the microbial inoculants and variety interaction on the number of medium tubers (Appendix 3: medium tubers).

All inoculated treatments and fertilized control had significantly higher tuber weight which was more than double that of unfertilized control (Figure 3.2).

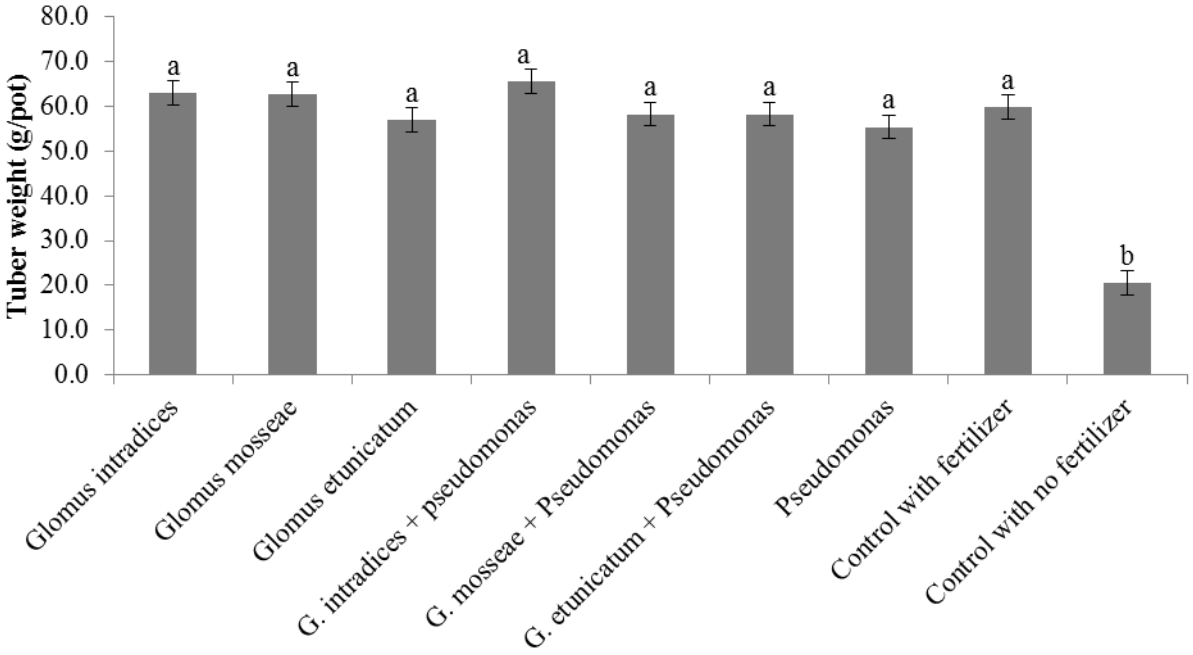


Figure 3.2: Tuber yield (g/pot) obtained from the potato inoculated with AMF and *Pseudomonas*. Bars indicate standard error bars. Bars with the same letter are not significantly different at P≤0.05

The highest shoot dry weight was 54% higher in Tigoni than Asante in duo inoculant of *G. intradices + Pseudomonas* (Table 3.2).

Table 3.2: Shoot biomass recorded in potato varieties inoculated with AMF and *Pseudomonas*

	Potato variety		
Treatment	Asante	Kenya Mpya	Tigoni
Glomus intradices	0.45cdef	0.55abcdef	0.58abcdef
Glomus mosseae	0.47bcdef	0.60abcde	0.57abcdef
Glomus etunicatum	0.49bcdef	0.62abcd	0.64ab
G. intradices + pseudomonas	0.44def	0.54abcdef	0.68a
G. mosseae + Pseudomonas	0.42ef	0.65ab	0.59abcdef
G. etunicatum + Pseudomonas	0.43ef	0.64abc	0.57abcdef
Pseudomonas	0.41f	0.60abcde	0.59abcdef
Control with fertilizer	0.54abcdef	0.63abcd	0.51abcdef
Control with no fertilizer	0.02g	0.13g	0.03g
Standard error	0.04		

Values transformed to $\log_{10}$ and are means of four determinations
Values with similar letters within a column are not significantly different at 5% level of significance

Kenya mpya had 1.5 times higher shoot weight than in cultivar Asante in duo inoculant of *G. mosseae* and *G. etunicatum* each with Pseudomonas while the lowest was in unfertilized control in all the cultivars.

Shoot phosphorus (P) content was in the order of Tigoni > Asante > Kenya Mpya (Table 3.3).

Table 3.3: Shoot phosphorus content µg/g with different inoculation regimes

Treatment	Potato Variety			
	Asante	Kenya Mpya	Tigoni	Means
Glomus intradices	1.08abcd	0.95cd	1.10abc	1.04b
Glomus mosseae	1.00bcd	1.00bcd	1.13abc	1.04b
Glomus etunicatum	1.13abc	1.00bcd	1.10abc	1.08b
G. intradices + pseudomonas	1.13abc	1.33ab	1.40a	1.29a
G. mosseae + Pseudomonas	0.98cd	1.15abc	1.00bcd	1.04b
G. etunicatum + Pseudomonas	1.10abc	1.00bcd	1.20abc	1.10b
Pseudomonas	1.10abc	0.95cd	0.98cd	1.01bc
Control with fertilizer	1.08abcd	0.90cd	0.93cd	1.00bc
Control with no fertilizer	0.93cd	0.75d	0.93cd	0.90c
Means	1.06ab	1.00b	1.08a	0.02
Standard error	0.06			

Values are means of four determinations
Values with similar letters within a column are not significantly different at 5% level of significance

Unfertilized control had the lowest P content of 0.8-0.9µg/g compared to the inoculated treatments in the three varieties that ranged from 1.0-1.4 µg/g. Compared with fertilized control, P uptake was only effective in Tigoni inoculated with *G. intradices + Pseudomonas* that had 1.5 times higher P content.

3.4.3 Mycorrhizal root colonization of potato roots after harvesting

Mycorrhizal colonization was observed in all treatments due to presence of arbuscules in the infected roots while there was no root colonization in the uninoculated-unfertilized controls. The highest percentage of root mycorrhizal colonization was mainly in Tigoni in all the AMF inoculated treatments especially in *G. etunicatum* with or without *Pseudomonas* which were over forty times that of fertilized control and Pseudomonas treatment of the same cultivar (Table 3.4).

Table 3.4: Root colonization (%) in potatoes seedlings inoculated with three AMFs and *Pseudomonas* isolate

Treatment	Potato Variety			
	Asante	Kenya Mpya	Tigoni	Means
Glomus intradices	5.0def	5.0def	15.3bcd	8.4c
Glomus mosseae	9.5bcdef	7.8cdef	15.8bcd	11.0bc
Glomus etunicatum	13.0bcdef	16.5abcd	21.5ab	17.0ab
G. intradices + pseudomonas	4.5def	10.0bcdef	14.0bcde	9.5c
G. mosseae + Pseudomonas	6.8def	11.0bcdef	20.3abc	12.5abc
G. etunicatum + Pseudomonas	13.0bcdef	14.0bcde	29.0a	18.7a
Pseudomonas	0.8f	1.8ef	0.5f	1.0d
Control with fertilizer	0.3f	0.3f	0.5f	0.3d
Control with no fertilizer	0.0f	0f	0.0f	0.0d
Standard error	2.42			1.4

Values are means of four determinations
Values with similar letters within a column are not significantly different at 5% level of significance

Tigoni had double root colonization compared to Asante and Kenya Mpya in duo inoculant of *G. etunicatum + Pseudomonas.* Among the treatments, *G. etunicatum+Pseudomonas* had the highest root colonization followed by single inoculant of *G. etunicatum* and duo inoculant of *G. mosseae+Pseudomonas.*

3.4.4 Mycorrhizal spore count from soil after harvesting

Identification of AMF was based on the morphological characteristic of the resting spores as suggested by INVAM (International Culture Collection of Arbuscular and Vesicular-Arbuscular Endomycorrhizal Fungi). *G. etunicatum* are orange to red brown in colour, *G. intradices* are white, pale cream to yellow brown sometimes with a green tint while *G. mosseae* are straw to dark orange-brown, but majority are yellow-brown any other spore apart from these colours was considered to be indigenous spore. There was significant variation among the treatment in the number of inoculated spores at harvest (Fig 3.3). All the treatments recorded higher number of inoculated spores with *Glomus mosseae + Pseudomonas* recording the highest number compared with *Glomus intradices*.

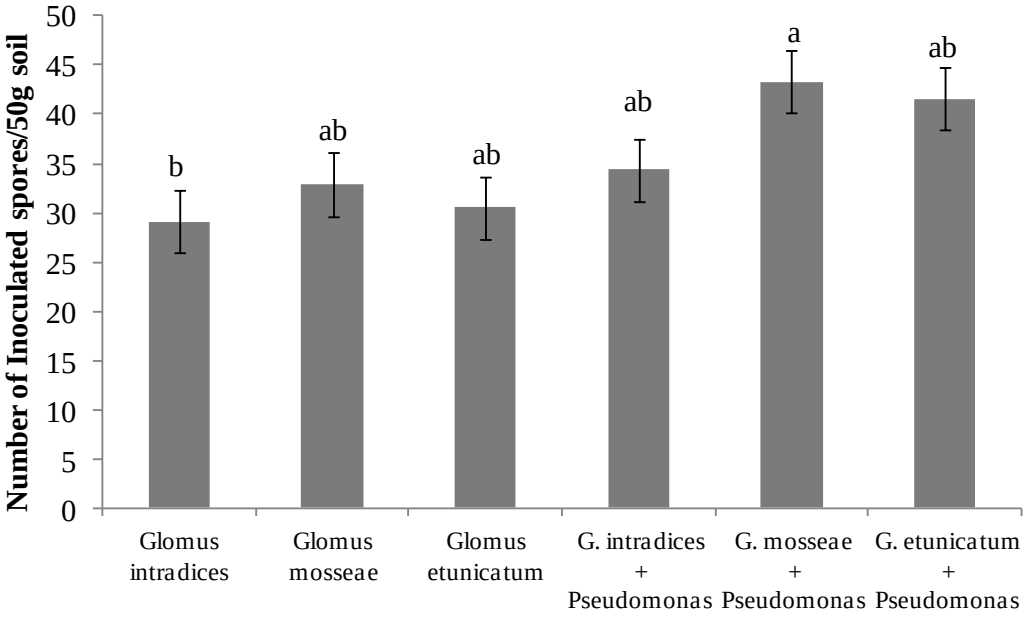


Figure 3.3: Variation in the number of spore among the inoculated treatments. Bars indicate standard error bars. Bars with the same letter are not significantly different at $P \leq 0.05$

However, there was no significant varietal effect on spore count (appendix 3: Inoculated spore count).

The number of indigenous spores was significantly higher in control with or without fertilizer and *Pseudomonas* inoculated treatment compared to all AMF inoculated treatments (Figure 3.4).

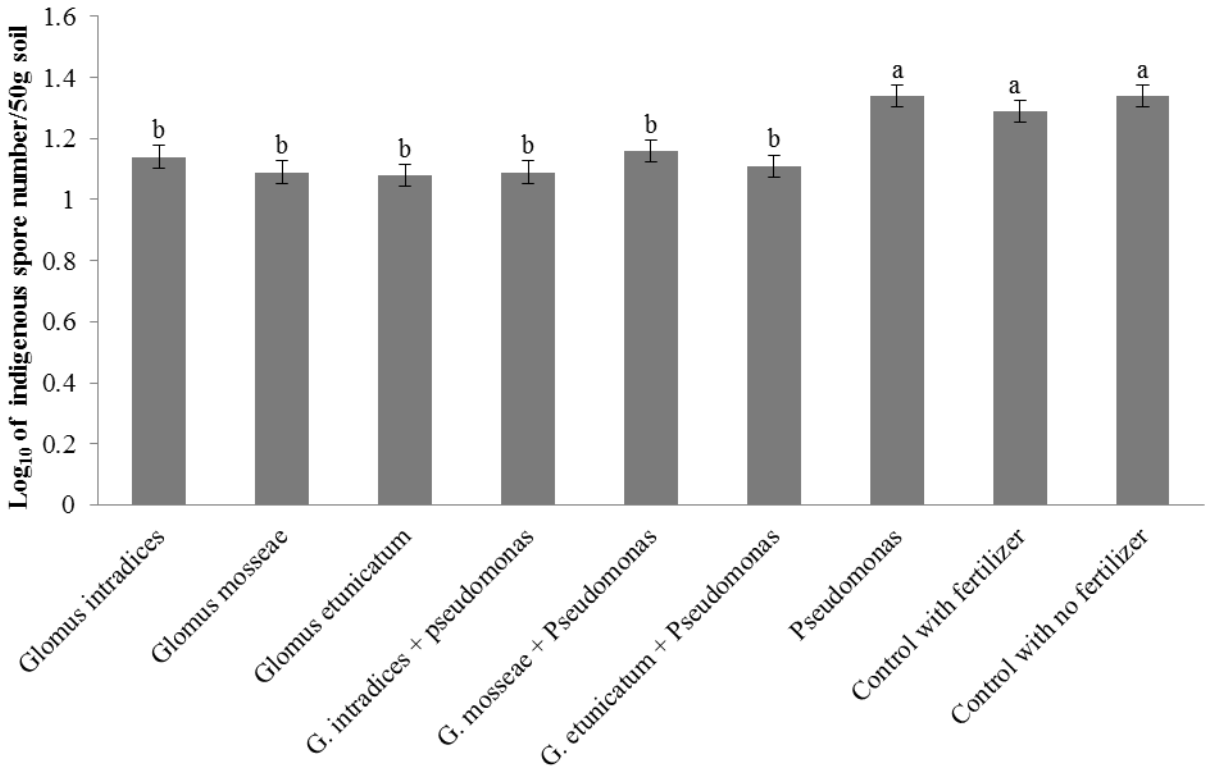


Figure 3.4: Indigenous spore number among the treatments in the soil after harvesting. Bars indicate standard error bars. Bars with the same letter are not significantly different at P≤0.05

There was positive correlation between inoculated spore count and total tuber weight (r=0.412), dry shoot weight (r=0.382) and Phosphorus (r=0.354). There was, however, a negative correlation between inoculated spore count and the indigenous spore count(r=-0.521). Inoculated spore count(r=0.571), total tuber weight (r=0.293), total tuber number(r=0.258), dry shoot weight(r=0.387) and phosphorus(r=0.362) were positively correlated to % mycorrhizal root colonization.

3.5 Discussion

3.5.1 Tuber grade, weight and shoot biomass as influenced by AMF and rhizobacteria inoculants

Unfertilized control generally had the least tuber numbers, weight and shoot biomass indicating the importance of fertilizer addition to augment the soil nutrient supply which when effectively absorbed

by the plants results in enhanced growth and increase in tuber yield as observed in fertilized control. On the other hand, there was no evidence of improvement in most of the microbial inoculants on shoot biomass, tuber number and yield when compared to fertilized control. This may be due to low AMF root colonization which ranged from 0 to 29% compared to 41-73% reported by Ngakou et. al., (2006) but under field conditions. Increased P in soil through addition of calcium nitrate based solution might have limited AMF root colonization. Tuber size is an important index of commercial value in potato seed production as it determines the number of eyes, sprouts and stem per tuber (Struik and Wiersema, 1999). High tuber weight and number of large and small size in some of the AMF inoculated treatments still indicate the potential of these microorganisms to enhance minituber production which is important in potato seed production (Duffy et. al., 2000). Potato can benefit from these beneficial microorganisms for nutrient uptake and growth by increasing the volume of soil explored, hence efficiency in nutrient uptake leading to increase in tuber yield (Davies et. al., 2005; Sieverding et. al., 1991). Enhanced shoot weight especially in duo inoculants of *G. intradices + Pseudomonas* indicates Pseudomonas played a role as mycorrhiza helper bacteria by stimulating spore germination, hyphal growth and mycorrhiza establishment (Garbaye, 1994) thereby enhancing nutrient uptake to the shoot. They are also known to produce antibodies against detrimental microorganisms that inhibit mycorrhizal symbiotic association with plants without inhibiting mycorrhiza formation and functioning (Vazquez et. al. 2000, Vessey, 2003).

3.5.2 Effect of microbial inoculants on P shoot content

It is well established that mycorrhizal fungi contribute significantly to P nutrition of plants; particularly under low P conditions (Barea et. al. 2008) as was the case of the soil used with only 12.4 mg/kg compared to the recommended range of 30-100mg/kg, according to Cropnut. But due to fertilizer addition that increased the P level in soil, the beneficia1 influence of AMF infection on the efficiency of plant P acquisition was absent in most of inoculated treatments resulting to equivalent P

shoot content as the fertilized-uninoculated control. This does not imply that the AMF inoculates were ineffective since all the shoot P in inoculated treatments might have essentially been provided solely by the mycorrhizal route of colonized roots after complete inactivation of the direct P uptake pathway via root hairs (Smith et. al. 2004). Furthermore, duo inoculant of *G. intradices* and *Pseudomonas* had the highest P content and this was due to positive interaction effect between the two microorganisms in which the P solubilized by the rhizobacteria is translocated to the plant shoot by the extrardial mycorrhizal mycelium ensuring efficient P utilization (Pavan Kumar Pindi, 2011). AMF and rhizobacteria therefore play a critical role of increasing fertilizer use efficiency (Lambers *et. al.*, 2009).

3.5.3 Mycorrhizal root colonization

Mycorrhizal root colonization rates differed among the treatments and this may be due to differences in genotypic makeup and host specificity of the AMF to potato (Duffy et. al., 2000). Enhanced root colonization especially in duo inoculants of *G. etunicatum+Pseudomonas* shows the positive interaction when AMF and rhizobacteria are added together to increase mycorrhizal root colonization (Sabannavar et. al., 2009; Johansson et. al., 2004). This may be due to phytohormones like Auxin secreted by the rhizobacteria that increases root proliferation and permeability enhancing plant susceptibility to mycorrhizal infection (Dobbelaere et. al. 2002; Erturk et. al., 2010). The native mycorrhiza may have not been effective in AMF uninoculated treatments due to low spore number and disturbance during sampling, handling and sieving of the soil when setting up of the experiment. This also shows that soil solarization usually inactivates the indigenous AMF reducing competition thereby enhancing root colonization by the inoculated strains (Ngakou et. al., 2006).

3.5.4 Inoculated and indigenous spores in soil

High inoculated spore number especially in *Glomus mosseae + Pseudomonas* shows that presence of rhizobacteria had stimulative effect on the spore number of mycorrhizal fungi (Sabannavar and

Lakshman, 2009). This may be through the production of phytohormones by rhizobacteria that enhance AMF colonization by increasing AMF hyphae penetration in the host plant (Toro et. al., 1997; Barea et. al., 2002).

AMF and introduced microbial inoculants affects the population of indigenous microbial communities in the plant rhizosphere as reported by Vazquez et. al. (2000), resulting to lower number of indigenous spore in AMF inoculated treatments. This may be attributed to high population of the inoculated microorganisms due to increased levels of viable spores and infected roots in the inoculum compared to the native microorganisms enabling them to compete effectively for plant nutrients in the rhizosphere (Bharadwaj, 2007). Presence of indigenous spore shows that soil-solarization does not destroy all soil microorganisms, but modifies the microbial balance in favour of the beneficial microorganisms (Mazzola 2004).

3.5.5 Conclusion

The results of the present study show that yield of potato can be improved by through a combination of an AM fungal symbiont and saprophytic *Pseudomonas*. The potential of using these microbial inoculations to enhance potato production in field condition and under different fertilizer regimes is important due to soil-plant-microbial complex interaction that influences the effectiveness of the inoculants.

CHAPTER FOUR

Performance of Rhizobacteria and Arbuscular Mycorrhiza Fungi (AMF) dual inoculants in control of Bacterial wilt (*Ralstonia solanacearum*) in Potato (*Solanum tuberosum*) production

4.1 Abstract

This study evaluated effects of three commercial strains of AMF; individually and in combination with antagonistic Rhizobacteria on bacterial wilt of potato. The treatments were: *Glomus intraradices, G. mosseae, G. etunicatum, G. intraradices + Pseudomonas, G. mosseae + Pseudomonas, G. etunicatum + Pseudomonas, G. intradices + Azoctobacter, G. mosseae + Azoctobacter, G. etunicatum + Azoctobacter, G. intradices + Bacillus, G. mosseae + Bacillus, G. etunicatum+ Bacillus*, positive control with fertilizer and negative control without fertilizer on two potato cultivars; Tigoni and clone (387164.4). Potatoes were planted four plants per crate in a complete randomized block design. The parameters evaluated include disease severity, latent infection, and tuber yield. Disease severity was more pronounced in Tigoni than in clone 387164.4 with or without microbial inoculation. There was 0% disease severity in duo inoculants containing *G. intradices + Pseudomonas* in clone 387164.4 and *G. etunicatum* and *G. intradices* both inoculated with *Bacillus* in both cultivars which all tested negative for latent infection. However, *G. etunicatum + Bacillus* in Tigoni with 0% disease severity tested positive for latent infection. *G. etunicatum+Bacillus* had the highest tuber weight, with 40% more than uninoculated and fertilized control. There was no variety treatment interaction on tuber number and nutrient uptake. Duo inoculants were effective in controlling bacterial wilt despite the low yield of potato.

Keywords; *Ralstonia solanacearum, Solanum tuberosum, G. intradices,* rhizobacteria, Disease severity.

4.2 Introduction

Potato is highly promoted for its important contribution towards food security and in combatting hunger and starvation especially in Asia and Africa. It is highly nutritious and compared to other food crops, it is highly profitable due to high yield per unit area within a short period (CIP, 1984). High incidences of bacterial and fungal diseases have limited potato production. Bacterial wilt also called brown rot of potatoes which is caused by race 3 biovar 2 of *Ralstonia solanacearum* is the second most important potato disease after late blight (Momol et. al., 2000; Felix et. al., 2010; Lemaga et. al., 2001) resulting to enormous losses of up to 100% under conditions favourable for disease manifestation (Boshou, 2005). The problem has been aggravated in Kenya due to limited availability of certified clean seeds resulting to use of infected potato tubers as planting materials by farmers leading to increase and spread of the disease (Kinyua et. al., 2001). Bacterial wilt was first reported in Kenya in 1940s and has spread to most potato growing regions ((Michieka, 1993). The bacterium which is endemic in the soil invades the root of the host plant and colonizes the xylem vessels causing irreversible wilting and death (Tahat et. al., 2008; Smith et. al., 1995). Disease control has been difficult due to the large number of hosts, high genetic and phenotypic variability of the pathogen, systemic localization of the pathogen, and lack of chemical control (Tahat et. al., 2008, Ji et. al., 2005, Hartman et. al., 1993). Use of resistant varieties under crop rotation is considered as efficient and cost effective alternative has limitation since response to bacterial wilt vary under different environments and also seasons within the same environment and can survive in the soil for twenty years in absence of the host plant (Felix et. al., 2010, Genin and Boucher, 2002). The need to search for alternative options in the management of the disease which are cost effective and eco-friendly is therefore critical.

Biological control of soil of borne diseases like bacterial wilt by introduction of antagonistic microorganisms is one such option (Raupach and Kloepper, 1998; Pal and Garedener, 2006) and this may be integrated with other conventional techniques in management of diseases. Rhizosphere

microorganisms such as arbuscular mycorrhiza fungi (AMF) and rhizobacteria have been shown to increase availability of plant nutrients such as Phosphorus (Idris et. al., 2009; Richardson et. al., 2009) through symbiotic association resulting to a strong healthy and vigorous plant increasing resistance to soil pathogens (Omorusi, 2005). AMF has been shown to increase tolerance of plants to soil pathogens by influencing the rhizosphere bacterial community altering their structure and function (Marschner et. al., 2001). Mycorrhizal fungi also compete for direct infection sites and change the root morphology by lignification of the cortical cell walls thereby inhibiting soil borne pathogens (Schenck, 1981, Vigo et. al., 2000). On the other hand, rhizobacteria act through aggressive proliferation and colonization of the rhizosphere thereby reducing the population of disease causing pathogens. Both AMF and rhizobacteria have been shown to produce antibiotics compounds that are antagonistic to pathogen development (Schenck, 1981; Richardson et. al., 2009; Ryan et. al., 2009). Since rhizobacteria and AMF are associated with plants roots, they are likely to interact in the rhizosphere resulting to positive synergistic effect which has the potential of improving agricultural production by enhancing plant growth, health and development, and also increase soil fertility (Lambers et. al., 2009) which is significant for a sustainable agriculture. The objective of the present study was to determine the efficacy of Arbuscular Mycorrhizal Fungi and rhizobacteria in controlling or suppressing disease development of bacterial wilt in potato.

4.3 Materials and Methods

4.3.1 Isolation of Bacterial wilt pathogen and inoculum preparation

From an infested potato field, newly diseased plant was identified and uprooted together with the tubers. Older diseased plants which are usually colonized by saprophytic fungi and bacteria which tend to grow quickly on isolation medium Casamino acids, Bacto-Peptone and Glucose (CPG) such that the bacterial wilt pathogen cannot be isolated were not used.

The plant roots were separated from the stem and washed thoroughly with water. Pathogenicity test using the vascular flow method was done. This involved cutting small portion of the stem 3-4cm from the plant base and suspending in vertical position in a clear test tube containing water and observing for the viscous white slime streams. The milky slimes exuded are a characteristic bacterial ooze of *Ralstonia solanacearum* from infected xylem. Positive tubers from the infected plant were washed, disinfected, cut and observed for bacterial exudate from the vascular ring.

The bacterial ooze was streaked on (CPG) medium as described by Kinyua et. al. (2012). After three days the petri dishes were examined for distinct bacterial wilt colonies and further multiplied in the same medium. After three days bacterial wilt colonies were made into two litre inoculum suspension with distilled water. The inoculum was thoroughly mixed and 1.5ml drawn to determine the population of bacterial wilt in the inoculum.

4.3.2 Bacterial wilt colony count

Bacterial colony count in the inoculum was determined using standard serial dilution plated on Semi-selective Media from South Africa (SMSA) and incubated at 30^0C for 48hrs. Colonies that showed typical *R. solanacearum* characteristics (fluidal and irregular with red or pinkish red centres and whitish periphery) were counted. Bacterial count of 10^{10}CFU/ml was observed.

4.3.3 Soil preparation and inoculation of bacterial wilt.

The experiment was conducted in a screen-house at Upper Kabete field station, University of Nairobi between September and December 2012. Soil used was sampled from a 15-year fallow plot at the National Potato Research Centre, KARI-Tigoni in Limuru. The Centre is about 40 km North-west of Nairobi city centre, at an altitude of 2131m ASL, latitude of 1°15' S and longitude 23° 46' E with humic nitosol or alfisol soil (Jaetzold et. al., 2006). Large soil clods and debris were removed by sieving the soil through 5mm mesh sieve, solarizated by covering with clear polythene sheet and left

exposed to the sun for thirty days (Pokharel, 2011). Sterilized coco peat was mixed with the soil in a ratio of 1:1 and was used as the growing media at a rate of 22 kg per crate. Potato varieties; Tigoni that is highly susceptible to Bacterial wilt, and clone (387164.4) that is considered less susceptible to bacterial wilt were used in this experiment. Well sprouted seed potatoes from International Potato Center (CIP) were planted in bread crates (9'H*21'L*16'W) lined with polythene bag. 10ml of bacterial wilt inoculum was applied in each crate and mixed evenly in 22kg of soil-cocopeat mixture, watered regularly and left for two weeks before planting with 4 well sprouted potato seeds.

4.3.4 AMF and rhizobacteria inoculation

The effect of soil inoculated with three AMF isolates individually and in combination with antagonist *Pseudomonas*, *Bacillius* and *Azoctobacter* against bacterial wilt was conducted in a screenhouse. Microbial inoculants were applied in four rows on the surface and approximately 5cm deep at the time of planting. About 5µl (10^8cfu/ml) of rhizobacteria isolates *Pseudomonas*, *Bacillus* and *Azoctobacter* and 25g of AMF supplied by Dudu-tech division of Finlays Horticulture, *G. intraradices (*AMF1), *G. mosseae (*AMF2), and *G. etunicatum* (AMF3) were applied in the rows according to the respective treatment except for the control which was not inoculated with the microbial inoculants. This experiment was arranged in randomized complete block design (RCBD) with four replicates in a factorial arrangement with variety and inoculums being the factors under consideration. DAP fertilizer (18-46-0) was applied at half the recommended rate of 250kg/ha in each case. The treatment were: *Glomus intraradices, G. mosseae, G. etunicatum, G. intraradices + Pseudomonas, G. mosseae + Pseudomonas, G. etunicatum + Pseudomonas, G. intradices + Azoctobacter, G. mosseae + Azoctobacter, G. etunicatum + Azoctobacter, G. intradices + Bacillus, G. mosseae + Bacillus, G. etunicatum+ Bacillus*, positive control with fertilizer and negative control without fertilizer on the two potato cultivars.

4.3.5 Observation of Bacterial wilt symptoms on potato

The plants were left for natural disease infestation and regularly watered to ensure that the soil was moist. Upon emergence the plants were rated visually for bacterial wilt severity every week from the third week to the ninth week of the experiment. A scale of 0-5 (from wilted leaves to death) was used where, 0 = no symptoms, 1 = 1 leaf wilted, 2 = 2 or 3 leaves wilted, 3 = all the leaves wilted except the top 2 or 3 leaves, 4 = all leaves wilted and 5 = plant dead, (Winstead and Kelman, 1952). Percent severity index (PSI) was calculated using the method described by Cooke (2006).

PSI = Σ (scores × 100)/ (number of plants rated × maximum scale of the scores) for each scoring date.

From the PSI, reaction to bacterial wilt was then calculated using the area under disease progress curve (AUDPC) as per the formula according to Campbell and Madden (1990):

$$\text{AUDPC} = \sum_{j=1}^{Nj-1} \left(\frac{y_j + y_{j+1}}{2}\right)(t_{j+1} - t_j)$$

where,

y_j = percentage (%) of bacterial wilt on the j^{th} observation,

t_j= the date of observation in days after planting and

n = the number of disease severity readings.

Harvesting of the tubers was done when 75% of the plants had reached senescence and tuber number and weight taken. Tuber grading was based on weight; <20g for small tubers and >20g for medium tubers. Tuber phosphorus content was determined using microwave-assisted acid extraction/dissolution of plant material (Laboratory test method, Crop Nutrition Laboratory services, 2013). Tubers were sampled for latent infection test (Priou et. al., 1999) using enzyme linked immunosorbent assay on nitrocellulose membrane (NCM-ELISA).

4.4 Data analysis and management

Data was subjected to analysis of variance (ANOVA) using proc GLM and least significant difference test for the variables conducted using Statistical Analysis System (SAS version 9). Due to non-homogeneity of variances data in N was square root transformed while Mn, B and Zn data were log transformed. Differences at $P \leq 0.05$ were considered significant.

4.5 Results

4.5.1 Characteristics of soil used in the experiment

Nutrient analysis of the soil used in the current study showed the following results; pH($CaCl_2$) 6.0, available phosphorus (Olsen) 12.4mg/kg, exchangeable cations 16.0 cmol/kg, and particle size distribution of 11.8%, 54% and 34.1% for silt, sand and clay, respectively.

4.5.2 Effects of rhizobacteria and AMF on disease severity and latent infection of potato tubers

Disease severity was more pronounced in Tigoni cultivar compared to clone across all treatments except in duo inoculants of *G. etunicatum* and *G. intradices* each inoculated with *Bacillus* having Area Under Disease Progression Curve (AUDPC) of zero in both cultivars (Figure 4.1).

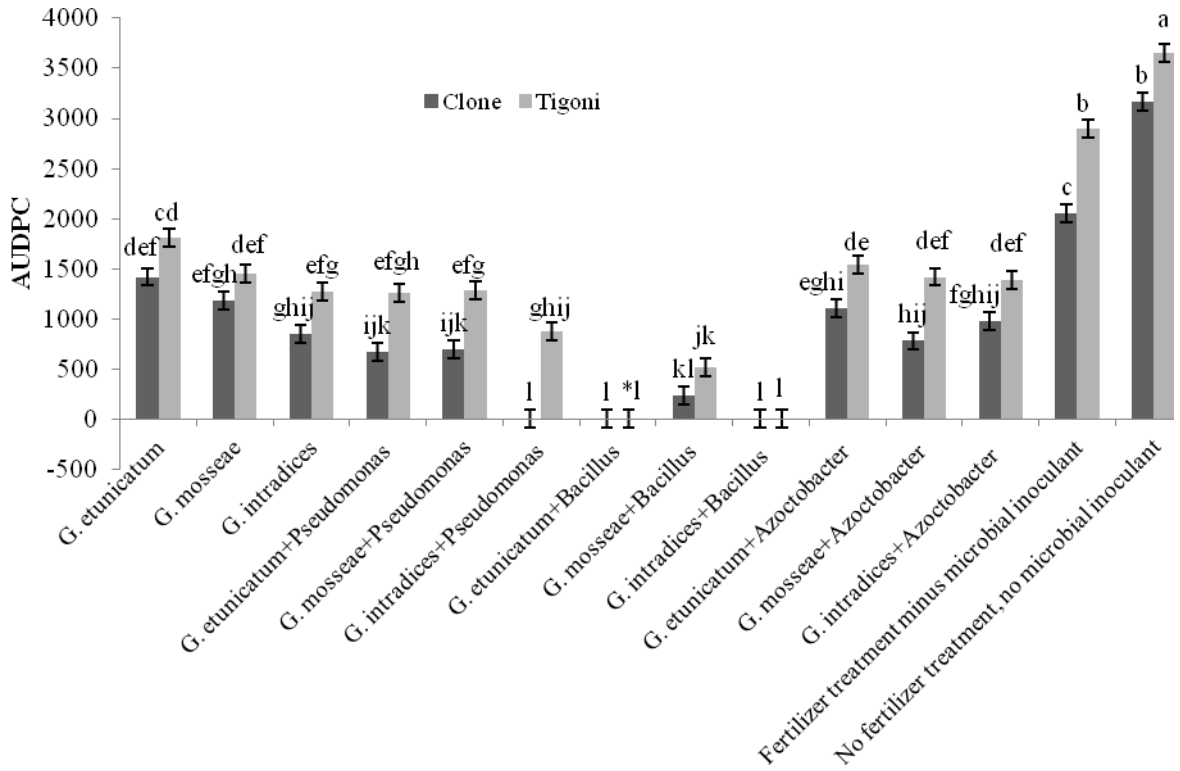


Figure 4.1: Area under disease progress curve for variety Tigoni and clone. Bars indicate standard error bars; Values with the same letter are not significantly different at P≤0.05

The bacterial wilt infection was highest in uninoculated unfertilized treatment followed by uninoculated fertilized treatment and *G. etunicatum*, respectively. Under *G. intradices +Pseudomonas,* Clone 387164.4 had zero AUDPC as opposed to Tigoni which had 871.

For the treatments that did not show disease symptoms; *G. intradices* + *Pseudomonas*, *G. etunicatum* + *Bacillus* for clone 387164.4 and *G. intradices* + *Bacillus* for both cultivars tested negative for latent infection using NCM-ELISA however, *G. etunicatum* + *Bacillus* in Tigoni tested positive (Table 4:1).

Table 4.1: Latent infection of tubers from bacterial wilt symptomless plants

Treatment	Cultivar	Latent infection
G. intradices + *Pseudomonas*	clone 387164.4	-
G. etunicatum + *Bacillus*	clone 387164.4	-
G. intradices + *Bacillus*	clone 387164.4	-
G. intradices + *Bacillus*	Tigoni	-
G. etunicatum + *Bacillus*	Tigoni	+

+/- denotes positive or negative for latent infection

4.5.3 Tuber grades and yield as influenced by AMF and rhizobacteria inoculants

All the microbial inoculated treatments and the fertilized control significantly increased the number of medium tubers compared to the unfertilized control that had the least tuber number (Figure 4.2).

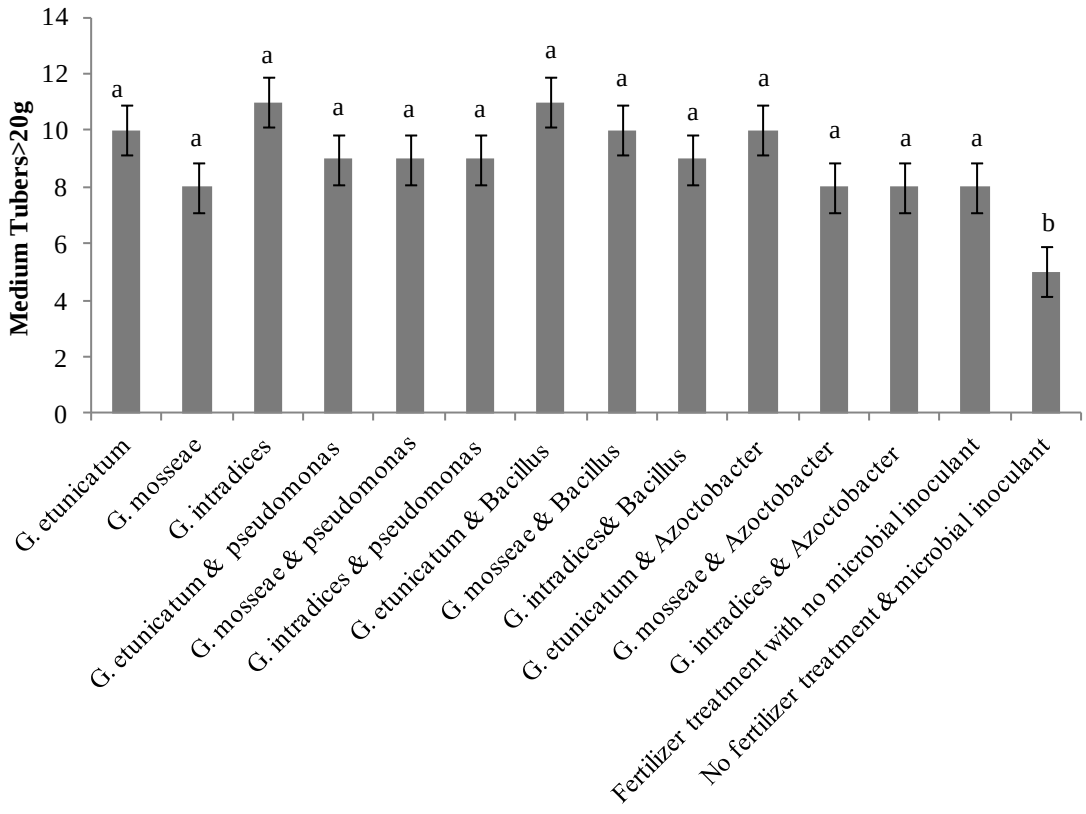


Figure 4.2: Number of medium tubers as influenced by AMF and rhizobacteria inoculants. Bars indicate standard error bars; Values with the same letter are not significantly different at P≤0.05

The effect of microbial inoculation was more pronounced in single inoculants of *G. etunicatum* and *G. intradices* and in duo inoculants of *G. etunicatum* and *G. mosseae* each combined with Bacillus and *G. etunicatum* and Azoctobacter. However, there was no effect of the microbial inoculants on number of small tubers on the variety, treatment and their interactions (appendix 4, small tubers).

There was significant effect in *G. intradices* with or without *Pseudomonas*, *G. etunicatum*+Pseudomonas, *G. etunicatum* and *G. mosseae* each inoculated with *Bacillus* on tuber weight compared to the uninoculated fertilized control (Figure 4.3).

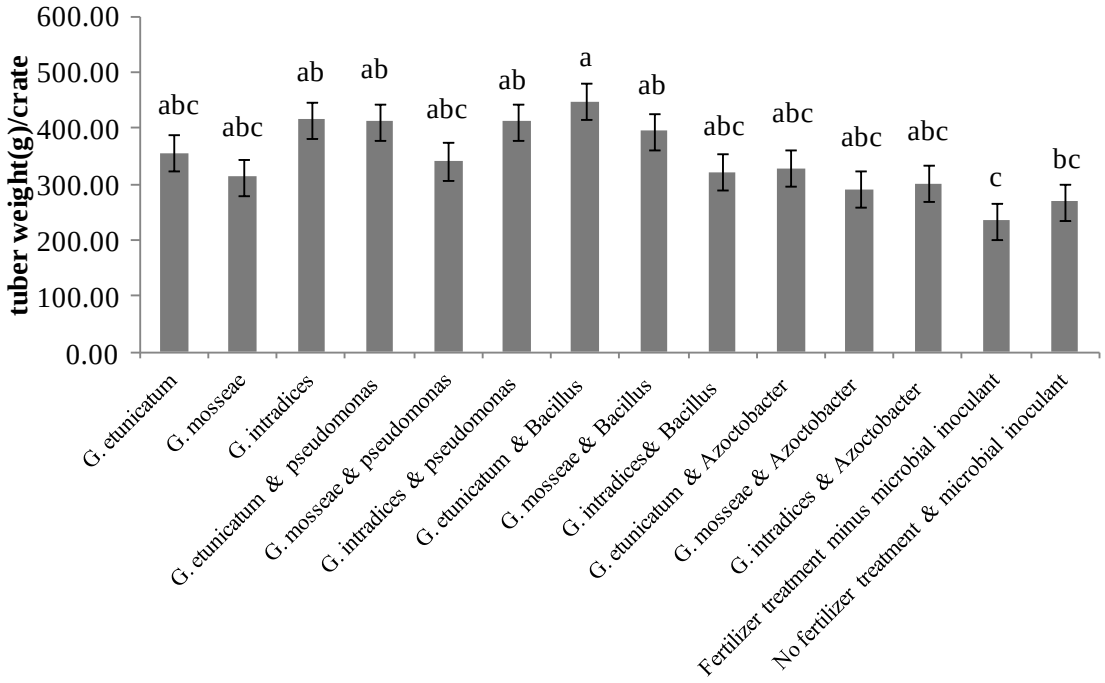


Figure 4.3: Variation in potato tuber weight under different inoculants of AMF and rhizobacteria. Bars indicate standard error bars; Values with the same letter are not significantly different at P≤0.05

G. etunicatum+Bacillus exhibited the highest tuber weight and this was 40% more than uninoculated and fertilized control. However, there was no variety treatment interaction on tuber weight (appendix 4, tuber weight)

4.5.4 Mycorrhizal root colonization and tuber minerals content

Root mycorrhizal colonization was observed in all the inoculated treatment, while there was no colonization in the uninoculated controls (Table 4.2). Among the inoculated treatments, 387164.4 had the highest root colonization with *G. intradices* and *Pseudomonas*, while the lowest was in Tigoni inoculated with *G. etunicatum.* In Tigoni the effect of duo inoculation was observed due to high rate of colonization compared to single AMF inoculants of *G. mosseae* and *G. etunicatum.*

Table 4.2: Root colonization (%) in potatoes seedlings inoculated with three AMFs and rhizobacteria strains

Inoculants	**Potato cultivars**	
	387164.4	**Tigoni**
G. etunicatum	24.3h	23.3h
G. mosseae	34.5efg	27.3gh
G. intradices	34.3efg	36.0cdef
G. etunicatum & *Pseudomonas*	38.5bcdef	33.0fg
G. mosseae & *Pseudomonas*	38.5bcdef	41.0abcde
G. intradices & *Pseudomonas*	48.8a	35.5def
G. etunicatum & *Bacillus*	43.8abc	41.8abcde
G. mosseae & *Bacillus*	45ab	42.0abcde
G. intradices & *Bacillus*	42.8abcd	43.0abcd
G. etunicatum & *Azoctobacter*	34.3efg	32.3fg
G. mosseae & *Azoctobacter*	37.5bcdef	34.8efg
G. intradices & *Azoctobacter*	39.0bcdef	37.0cdef
Fertilizer treatment with no microbial inoculant	0i	0i
No fertilizer treatment & microbial inoculant	0i	0i
Means	32.93	30.48
Standard Error	1.43	

Values are means of four determinations
Values with similar letters within a column are not significantly different at 5% level of significance

The effect of microbial inoculants on tuber nutrient content was only observed at the variety level with variety Tigoni recording 15%N, 26%P, 14%K and 40% Cu higher than clone 387164.4 (Table 4.3). However, Fe was 15% higher in clone 387164.4 than Tigoni variety.

Table 4.3: Mineral element contents of Tigoni and Clone 387164.4 in different inoculants of AMF and rhizobacteria

Element	Potato cultivar	
	Tigoni	Clone
% Nitrogen	1.24a	1.06b
% Phosphorus	0.19a	0.14b
% Potassium	2.62a	2.26b
Copper (ppm)	6.66a	3.97b
Iron (ppm)	121.64b	143.76a

Values are means of four determinations
Values with similar letters in the same row are not significantly different at 5% level of significance

4.6 DISCUSSION

4.6.1 Disease severity and latent infection of potato

Microbial inoculates of AMF and rhizobacteria suppressed the bacterial wilt disease compared to the control and this was mainly in clone 387164.4 which implies that AMF inoculation could suppress bacterial wilt infection and co-inoculation with rhizobacteria was more effective since *G. intradices* and *Pseudomonas* for clone 387164.4, *G. intradices* and *Bacillus* and *G. etunicatum* and *Bacillus* for both cultivars did not exhibit any disease symptoms. This confirms that dual inoculation of AMF and rhizobacteria has synergistic beneficial effect as biocontrol since each of the microbes have different biological functionings in the soil and when combined they confer a positive interaction that improves the health of plants. The two cultivars varied in their response to bacterial wilt infection with Tigoni being highly affected. This shows that different cultivars vary in their response to bacterial wilt within the same environment (Felix et. al., 2010).

Both cultivars in most of the inoculated treatment exhibited bacterial wilt symptoms indicating that the pathogenic strain was highly aggressive. The effectiveness of microbial inoculants also depends on the time and method of inoculation. Tahat et. al. (2012) applied the pathogen one month after the inoculate which was evenly mixed in the soil resulting to low disease severity in inoculated treatments. This is in contrast with this experiment where the microbial inoculants were applied two weeks after the

bacterial wilt inoculum in rows 5cm into the soil. This might have limited the inoculate colonization and establishment thereby reducing the plants ability to resist bacterial wilt attack. Based on the results of the experiment, it can be deduced that there was pathogen infection before the establishment of the microbial inoculates due to earlier inoculation of bacterial wilt and preventing pathogen development after infection was difficult (Eduardo et. al., 2007). This is especially so considering that *R. solanacearum* has the potential of producing bacteriocins that can inhibit AMF spore germination as observed by Tahat (2008a). The strategic means of controlling bacterial wilt is preventing pathogenic infection. The mode of action by microorganism should therefore begin at the early stage of host-pathogen interaction (Eduardo et. al., 2007), showing the importance of early inoculation of potato seeds prior to inoculation with pathogen. This ensures competitive colonization and establishment of microbial inoculants in the root zone, a prerequisite for effective biocontrol, considering that the first step in controlling pathogenic soil borne microorganisms is efficient rhizosphere colonization by the applied inoculants (Gao et. al., 2012). Furthermore, antagonistic or competing microorganisms which might have been present in the soil might have affected the survival rate of the inoculants reducing their population. This shows that the interaction between the native and introduced microorganisms play a significant role in disease control and is a challenge that is difficult to control (Eduardo et. al., 2007). The effectiveness of microbial inoculants therefore depends on their adaption to the soil where they have been introduced.

G. etunicatum and *Bacillus* for Tigoni cultivar did not show disease symptoms but tested positive for latent infection. This shows that a plant may appear healthy since they are able to tolerate development of visible bacterial wilt symptoms but are carriers of the disease which manifest later in the tubers. Unlike Tigoni, all treatments for 387164.4 that did not exhibit disease symptoms tested negative for latent infection. This is in agreement with Jill et. al. (2004) who found that tubers of tolerant potato plants are less likely to be latently infected. The varied response of these microbial inoculants shows

the importance of testing them in the field. This is because according to Azcón-Aguilar and Barea (1996), the biocontrol effectiveness of these beneficial microorganisms are difficult to conclude due complexity of the microbe, soil, plant system and environmental condition influence. It is therefore critical to identify the right combination factors so as to fully exploit their effectiveness.

4.6.2 Potato tuber grading and weight and root colonization as affected by AMF and rhizobacteria inoculants

There was no significant difference between the uninoculated controls and most of the inoculated treatments. This indicates that the *Ralstonia solanacearum* bacterium was highly virulent with advanced effect on potato productivity resulting to reduced tuber number and weight. This is contrary to expectation that reduction in disease severity would result in increased plant productivity (Gado 2013).

The results of mycorrhizal root colonization show that both cultivars of potato responded well to AMF inoculation in the presence of *R. solanacearum*. Single AMF had high root colonization but this was further improved in the presence of rhizobacteria signifying that rhizobacteria do not inhibit but enhance mycorrhizal colonization of the roots by producing phytohormones and are best referred to as mycorrhizal helpers confirming the findings by Azcon et. al., (1978). Clone 387164.4 had relatively higher root colonization therefore able to reduce bacterial wilt severity than Tigoni. This links the bioprotection effect of AMF on the level of root colonization (Khaosaad et. al. 2007), implying that a high degree of AM root colonization leads to high localized bioprotective effect. This also indicates that different plant cultivar respond differently to microbial inoculates and through root exudate secretion are able to therefore influence the soil microbial community (Garbeva et. al., 2004).

Tigoni recorded higher mineral content than Clone 387164.4 except for Fe indicating that tuber mineral concentration varies with the potato cultivar (White et. al., 2009). The microbial inoculants

did not enhance nutrient uptake. This may be due to AMF inability to build a strong hyphal network to increase nutrient absorption due to the pathogen inhibitory effect (Tahat et. al., 2008b). This suggests that plant nutrient status is not the main mechanism of inhibition effect of AMF and rhizobacteria on bacterial wilt infection.

4.6.3 Conclusion

The study indicates that, AMF and rhizobacteria inoculants were effective in the control of bacterial wilt of potato although, this did not result in higher productivity. This shows the economic importance of the disease. Tubers must be tested for latent bacterial wilt infection for symptomless plants grown in bacterial wilt infested soil. However, as with all attempts with biological control, more trials should be undertaken especially under field conditions to confirm their effectiveness considering variability in the native soil fauna, flora and microorganisms, that may militate the success of the inoculants and reveal different responses on the efficiency of AMF and rhizobacteria inoculants in control of diseases especially bacterial wilt.

CHAPTER FIVE

Application of Rhizobacteria inoculants and organic amendments in suppression of Bacterial Wilt (*Ralstonia solanacearum*) in Potato (*Solanum tuberosum*) production

5.1 Abstract

Bacterial wilt is an important soil borne disease that greatly affects potato production whose control has been difficult due to lack of chemical control. Use of antagonistic microorganisms is gaining interest due to their significant role in suppressing pathogenic infection. The aim of this study was to evaluate the effectiveness of three rhizobacteria isolates and organic amendments on control of bacterial wilt on two potato cultivars; Tigoni and clone (387164.4). There treatments were; *Pseudomonas*, *Bacillus*, *Azoctobacter*, *Pseudomonas+Bacillus*, *Pseudomonas+Azoctobacter*, *Bacillus+ Azoctobacter*, *Pseudomonas+Bacillus+Azoctobacter*, cattle manure minus microbial inoculant, poultry manure minus microbial inoculant, compost minus microbial inoculant and control. Potatoes were planted in complete randomized block design, four plants per crate. All microbial inoculates and poultry manure suppressed bacterial wilt infection mainly in triple inoculation of *Pseudomonas+Bacillus+Azoctobacte*r that also had the highest tuber weight and tested negative for latent infection in clone 387164.4. There was no effect of the microbial inoculants and organic amendments on small and medium tuber grading and on tuber phosphorus content. These microbial inoculants were effective as biocontrols, although further field studies are needed to determine their response under different soil conditions.

Key words: *Solanum tuberosum*, *Ralstonia solanacearum*, *Pseudomonas*, *Bacillus*, *Azoctobacter*

5.2 Introduction

Bacterial wilt caused by *Ralstonia solanacearum* is the second major constraint to potato production worldwide. This is because it is soil borne devastating systemic plant disease having a wide range of host resulting to enormous agricultural losses (Hayward, 1991). The pathogen penetrates the plants roots through wounds or natural openings and attacks the vascular system of the plant inhibiting nutrient and water translocation resulting to wilt, collapse and eventual death of plant (Kinyua and Miller, 2012). They are more recalcitrant to management due to lack of effective chemical control. The best control measures rely on use of resistant varieties and crop rotation. However, this has limitations due to ability of the pathogen to survive saprophytically in soil for even twenty years without the presence of susceptible host and response of potato varieties under different environment and seasons (Felix et. al., 2010; Genin and Boucher, 2002). Integrated and sustainable disease management options are therefore needed to control this highly destructive and challenging disease. Use of organic amendments and biocontrols are considered effective in suppressing pathogenic diseases, and the need to prevent environmental pollution by replacing or reducing the use of agrochemicals. The beneficial microorganisms include mycorrhizal fungi and antagonistic rhizobacteria such *Bacillus* spp., *Pseudomonas* spp. and *Azoctobacter* spp. (Pozo et. al., 2008; Aliye et. al., 2008; Lemessa et. al., 2007). Organic amendments enhances the natural suppressiveness of the soil by increasing the number of antagonistic microorganisms against soil-borne pathogens (Veeken et. al., 2005).

These root colonizer bacteria exert beneficial effect on the plants and are thus known as Plant Growth Promoting rhizobacteria (PGPR) as they act as biofertilizers and biocontrols (Jetiyanon and Kloepper, 2002). They are aggressive and persistant root colonizers and able to survive until maturity of the cultivated crop (Kloepper et. al., 1980, Bahme et. al., 1987). Rhizobacteria genera of *Bacillus* and

Pseudomonas are considered as powerful phosphate solubilizers (Rodríguez and Fraga 1999) that mineralize Phosphorus through production of organic acids and phosphatase enzyme improving the nutrient status of the plant. Furthermore, some like the *Bacillus* are able to form endospores while *Pseudomonas* are highly versatile, able to survive under adverse environmental conditions due to their low nutrient requirement and are effective against a wide range of pathogens in various plant species (Van Loon et. al., 1998; Antoun and Prévost, 2005; Mercado-Blanco and Bakker et. al., 2007). A single PGPR often have multiple modes of action of biological control (Kloepper, 2003; Vessey, 2003).Their high rate of root colonization enables them to compete effectively for nutrient resources resulting to exclusion of pathogens in the rhizosphere. They are also known to induce systemic resistance by stimulating the plant's natural defense and form biofilms on plant root hence confering protection on the host plant (Ongena and Jacques, 2008, Morris and Monier 2003). They produce antibacterial metabolites that are antagonistic to the phytopathogens that inhibit their mobility and growth reducing their deleterious effect on crops (Cazorla et. al., 2007; Touré et. al., 2004) and reduce plant root membrane penetration to pathogenic infection (Bashan et. al., 1991). Siderophores secreted have also been shown to limit Fe availability needed for growth and activity of pathogen (Kloepper et. al., 1980). The objective of the present study was to determine the efficiency of rhizobacteria in controlling or suppressing disease development of bacterial wilt in potato.

5.3 Materials and Methods

5.3.1 Isolation of Bacterial wilt pathogen and inoculum preparation

From an infested potato field, newly disease plants were identified and uprooted together with the tubers. Older diseased plants were not used as they are known to be colonized by saprophytic fungi and bacteria which tend to grow quickly on isolation medium Casamino acids, Bacto-Peptone and Glucose (CPG) so that the bacterial wilt pathogen cannot be isolated.

The plant roots and the stem base were cut and washed thoroughly with water. Pathogenicity test was undertaken by cutting small portion of the stem near the base of the plant and dipped in water in a clear test tube and observed for the viscous white slime streams. The threads are bacterial ooze from infected xylem. Positive tubers from the infected plant were washed and sterilize cut and the vascular ring inside of the tuber observed for bacterial slime oozing.

The bacterial ooze was streaked on (CPG) medium described by Kinyua et. al., 2012. After 3 days the petri dishes were examined for distinct bacterial wilt colonies and further multiplied in the same medium. After three days the bacterial wilt colonies were made into two liter inoculum suspension with distilled water. The inoculum was thoroughly mixed and 1.5ml drawn to determine the population of bacterial wilt in the inoculum.

5.3.2 Bacterial wilt colony count

Serial dilution 10 fold six times the inoculum suspension was prepared then 10^{-3}, 10^{-4} and 10^{-5} were plated in petri dishes in Semi-selective media of South Africa (SMSA) media in duplicates and incubated at 30 ^{0}C for 48hrs plated in duplicates and incubated at 30^{0}C for 48hrs. Colonies that showed typical *R. solanacearum* characteristics (fluidal and irregular with red or pinkish red centres and whitish periphery) were counted. The bacterial count in the inoculum was 10^{10}CFU/g

5.3.3 Soil preparation and inoculation of bacterial wilt

The experiment was conducted in a screen-house at Upper Kabete field station, University of Nairobi between September and December 2012. Soil used in this experiment was sampled National Potato Research Centre at KARI-Tigoni in Limuru, from a plot uncultivated for 15 years. The Centre is about 40 km North-west of Nairobi city centre, with altitude of 2131m ASL, latitude of 1°15' S and longitude 23° 46' E with humic nitosol or alfisol soil (Jaetzold et. al., 2006). Nutrient analysis of the soil showed the following results; pH ($CaCl_2$) 6.0, available phosphorus (Olsen) 12.4mg/kg,

Exchangeable cations 16.0 cmol/kg, and particle size distribution of 11.8%, 54% and 34.1% for silt, sand and clay respectively. The soil was sieved through 5mm sieve to remove large soil clods and debris and solarized by covering the soil with polythene sheet and left exposed to the sun for one month (Ramesh Pokharel, 2011). Sterilized coco peat was mixed with the soil into the ratio of 1:1 which was used in the screen house as the growing media. Two potato varieties namely; Tigoni that is highly susceptible to Bacterial wilt and a clone (387164.4) that is considered less susceptible to bacterial wilt were used in these experiments. Well sprouted potato seeds were obtained from International Center for potato (CIP) and planted in bread crates lined with polythene bag. Each crate had 10ml of bacterial wilt inoculum evenly mixed in 22kg of soil-cocopeat mixture, watered regularly and left for two weeks before planting with 4 well sprouted potato seeds.

5.3.4 Rhizobacteria inoculation

Microbial inoculants were applied in four rows on the surface and 5cm deep at the time of planting. About 5µl (10^8cfu/ml) of rhizobacteria strain *Pseudomonas*, *Bacillus* and *Azoctobacter* were applied individually, double and triplicate combination according to their respective treatments in each of the row except for the control and three organic amendments which were not inoculated with the microbial inoculants making 11 treatments namely: *Pseudomonas*, *Bacillus*, *Azoctobacter*, *Pseudomonas+Bacillus,Pseudomonas+Azoctobacter,Bacillus+Azoctobacter,Pseudomonas+Bacillus+Azoctobacter*, Cattle manure minus microbial inoculant, Poultry manure minus microbial inoculant, Compost minus microbial inoculant and Control.

Each of the treatments were replicated four times each established bread crate (9'H*21'L*16'W) containing 4 plants in a randomized complete block design with factorial arrangement having variety, microbial inoculants and organic amendments as factors under consideration. DAP fertilizer (18-46-0) was applied at 250kg/ha in all the inoculated treatments so as to enhance the effectiveness of microbial

inoculants which is increased under low soil fertility. Cow manure, poultry manure and compost were each applied at the rate of 10 tonnes/ha.

5.3.5 Observation of Bacterial wilt symptoms on potato

The plants were left for natural disease infestation and watered regularly to ensure that the soil was moist. Upon emergence the plants were rated for bacterial wilt severity on a weekly basis up to 30 days after inoculation. A scale of 0-5 (from wilted leaves to death) was used where, 0 = no symptoms, 1 = 1 leaf wilted, 2 = 2 or 3 leaves wilted, 3 = all the leaves wilted except the top 2 or 3 leaves, 4 = all leaves wilted, 5 = plant dead, (Winstead and Kelman, 1952).

Percent severity index (PSI) was calculated using the method described by Cooke (2006) using the formula;

PSI = Σ (scores × 100)/ (number of plants rated × maximum scale of the scores) for each scoring date.

From the PSI, reaction to bacterial wilt was calculated using the area under disease progress curve (AUDPC) according to Campbell and Madden (1990) formula:

$$\text{AUDPC} = \sum_{j=1}^{Nj-1} \left(\frac{y_j + y_{j+1}}{2}\right)(t_{j+1} - t_j)$$

where,

y_j = percentage (%) of bacterial wilt on the i^{th} observation,

t_j= the date of observation in days after planting and

n = the number of disease severity readings.

Harvesting of the tubers was done when 75% of the plants had reached senescence and tuber number and weight taken. Tuber grading was based on weight; <20g for small tubers and >20g for medium tubers. Tubers from plants were then sampled for latent infection test (Priou et. al., 1999) using enzyme linked immunosorbent assay on nitrocellulose membrane (NCM-ELISA). Tuber phosphorus

content was determined using microwave-assisted acid extraction/dissolution of plant material (Laboratory test method, Crop Nutrition Laboratory Services, 2013).

5.3.6 Data analysis

Data was subjected to analysis of variance (ANOVA) using proc GLM, least significant difference test for the variables conducted using Statistical Analysis System (SAS version 9). Differences at $P \leq 0.05$ were considered significant.

5.4 Results

5.4.1 Chemical characteristic of organic materials (amendment)

Despite its alkalinity poultry manure had the highest level of nutrients compared to compost and cattle manure (table 5.1). The compost manure however, had highest while poultry manure had the lowest organic matter content.

Table 5.1: Characteristics of organic amendments

Nurient element	Compost manure	Cattle manure	Poultry manure
pH	6.3	8.3	8.7
C/N ratio	18.3	16	14.4
Phosphorus %	0.41	0.22	1.64
Potassium %	0.63	1.54	2.74
Calcium %	1.46	1.28	6.16
Magnesium ppm	0.28	0.32	0.94

5.4.2 Effects of Rhizobacteria on disease severity and latent infection of potato tubers

Treatments with low AUDPC are considered to be effective in suppressing bacterial wilt while those with higher values being less effective (figure 5.1)

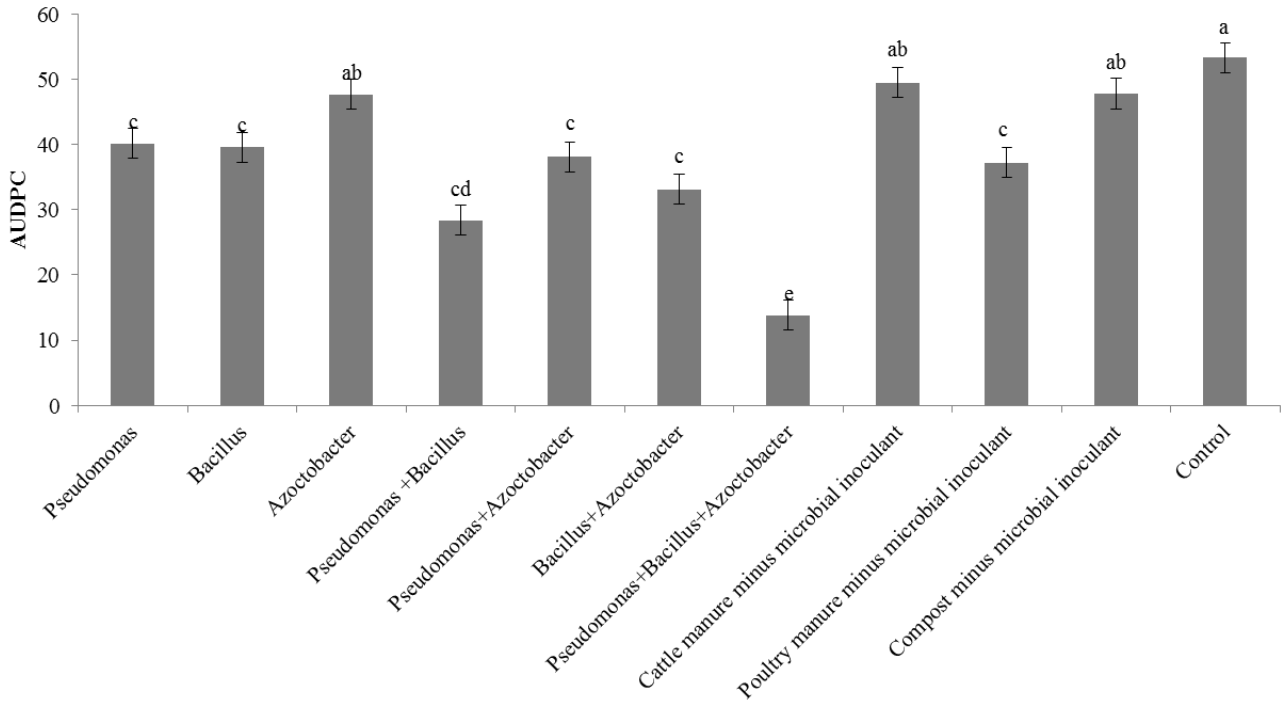


Figure 5.1: Area under disease progress curve for various microbial and organic treatments. Bars indicate standard error bars. Values with the same letter are not significantly different at P≤0.05

Microbial inoculation and poultry manure suppressed bacterial wilt infection. Triple inoculation of *Pseudomonas+Bacillus+Azoctobacter* had the highest level of bacterial wilt suppression. Bacterial wilt infection was more pronounced in *Azoctobacter*,organic amendments of compost and cattle manure and control. Among the inoculated treatments, rhizobacteria combination was more effective than single inoculation in suppressing infection. It was only mixed inoculum of *Pseudomonas* + *Bacillus* + *Azoctobacter* in clone 387164.4 that tested negative for latent infection.

5.4.2 Tuber grading and yield as influenced by Rhizobacteria inoculants

Tuber yield was highest in duo inoculants of *Pseudomonas+Azoctobacter*, *Bacillus+Azoctobacter* and in triple inoculant of *Pseudomonas+Bacillus+Azoctobacter* compared to uninoculated compost (figure 5.2).

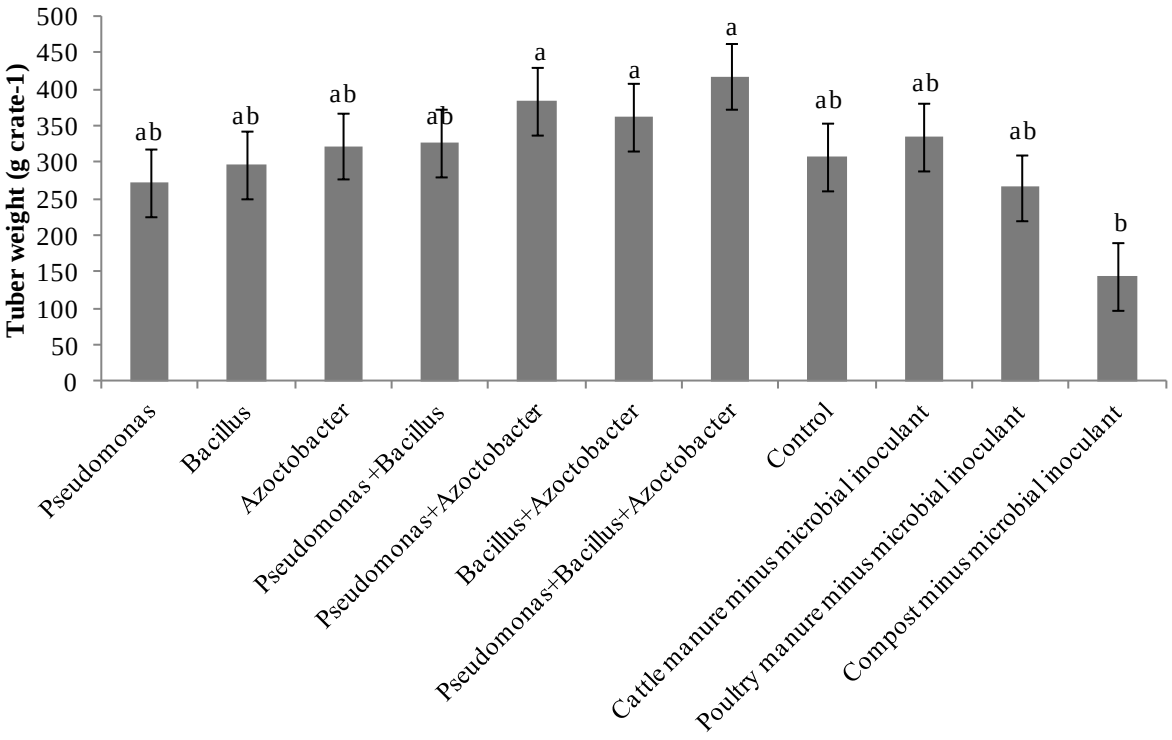


Figure 5.2: Effect of rhizobacteria inoculants on potato tuber yield. The bars indicate standard error bars. Values with the same letter are not significantly different at P≤0.05

There was no effect of the microbial inoculants and organic amendments on variety and their interactions on small and medium tuber grading and on tuber phosphorus content.

5.5 Discussion

5.5.1 Disease severity and latent infection of potato

The longer the plant takes to be infected by the pathogen, the higher the level of tolerance (Felix et. al., 2010). Rhizobacteria inoculation was effective in reducing bacterial wilt symptoms in potato in duo and triple combination. This can be attributed to the synergistic effect of the antagonistic inoculates of *Pseudomonas*, *Azoctobacter* and *Bacillus* resulting into a complex interaction which reduced the damage that might have been caused by the pathogen (Trotta et. al., 1996). Combining antagonists with different mode of action can therefore be effective in disease control (van Lenteren, 2000). Lozoya-Saldana et. al. (2006) found that a mix of strains of pseudomonas was effective in control of

late blight of potato. Single inoculate of rhizobacteria *Azoctobacter* was inefficient in controlling bacterial wilt and this may be due to the individual isolate showing low biological activity (Rajkumar et. al. 2005) thereby unable to prevent pathogenic attack or reduce their population. This confirms the findings by Lemancea and Alabouvette (1991), who observed that some Plant growth promoting rhizobacteria (PGPR) were not effective antagonists of pathogens when applied alone. The competitive ability of a biocontrol isolate partly is determined by its capacity to establish in soil and in the plant rhizosphere, and is probably involved in its capability to colonize the root surface. They are considered to be more effective as biocontrols when inoculated on the seed before planting or directly in the vascular system of the plant, as they are able to establish themselves more on the crop roots (Kempe and Sequeira 1983; Saharan et. al., 2011) a prerequisite for their effective use. This may also be due to the presence of competitive and deleterious microorganisms that inhibited the biocontrol activities of these individual strains (Kropp et. al., 1996). The different response of potato to rhizobacteria inoculation also depends on the bacterial isolate used. Poultry manure reduced disease severity than some of the inoculated treatments. This can be attributed to the amendment having high nutrients providing favourable conditions for proliferation of native antagonists microbes in the soil which were otherwise effective as biocontrol (Larkins 2008). The alkalinity of poultry manure might have inhibited pathogen growth which prefers acidic conditions (Fajinmi et. al 2010). Solarization does not destroy all soil microorganisms, but modifies the microbial balance in favour of the beneficial microorganisms (Raaijmakers et. al., 2009). Combining organic amendments and soil solarization have also been shown to result in accumulation of ammonium or ammonia in the soil which reduces the inoculum densities (Ndiaye et. al. 2007; Oka et. al. 2007). Native microflora also plays a critical role in determining the microbial inoculate survival in the soil (Singh et. al., 2000). For these microorganisms to be beneficial, they need to be available in high number than that of the pathogen to achieve control and well distributed in the host tissue to be able to effectively trigger the defense

response of the plant (Eduardo et. al., 2007; Saharan et. al., 2011). Therefore, an increase in the concentration of each rhizobacteria inoculant may eventually lead to better disease control. This is however, critical during the initial stage of microbial inoculation since the bacterial population tend to decline progressively immediately after inoculation which may prevent their population buildup to effect plants' tolerance to pathogenic infection (Bashan and Levanony, 1988; Yoav Bashan, 1998).

5.5.2 Potato tuber grades, weight and Phosphorus content

Increase in tuber weight in the inoculated and organic treatments despite disease infestation shows that the introduced microbial inoculants acted as plant growth promoting bacteria, able to produce hormones that increase root proliferation thereby enhancing nutrient uptake and plant vigour (Glick et. al. 1995, Gupta et. al. 2002) and increased proliferation of beneficial microorganisms native to the soil in the organic amendments that acted as biofertilizers that enhance growth and productivity of the plant (Larkins 2008). High tuber weight in the mixed inoculants especially in triple inoculation of *Pseudomonas, Azoctobacter* and *Bacillus* indicate the importance of multiple inoculations to enhance crop productivity. However, due to disease related stress the tuber number and phosphorus uptake was not influenced by the microbial inoculates and organic amendments signifying the great yield losses due to bacterial wilt on potato production. Increase in host plant tolerance to pathogenic attack has been attributed to enhanced P uptake. This is an unlikely mechanism in this case, since there was no significant difference in P uptake in the inoculated treatments and the control which had the highest level of infection. The involvement of other mechanisms such as morphophysiological or biochemical changes may have been involved.

5.6 Conclusion

The study indicates that:

i) Low yield despite fertilizer and rhizobacteria inoculation shows bacterial wilt disease as a major threat to potato production.

ii) Mixtures of rhizobacteria are better inoculum than individual rhizobacteria inoculation.

iii) Rhizobacteria and organic amendments were effective in suppressing bacterial wilt especially in mixed inoculum and in poultry manure. However, the method and timing of inoculation also seem to be an influential factor which should be further researched on especially under field conditions.

CHAPTER SIX

Assess performance of potato (*Solanum tuberosum*) inoculated with Arbuscular Mycorrhizal Fungi (AMF) under Aeroponic System

6.1 Abstract

Potato plants are relatively inefficient at acquiring soil Phosphorus (P) which is further aggravated by the low P solubility forms of phosphates of Fe, Al and Ca. This problem is overcomed in aeroponics soiless system and increases yield of disease free seed-tubers.Application of biofertilizers like AMF has been known to enhance the crop productivity, nutrition and reduce fertilizers and pesticides inputs through the extended mycorrhizal hyphae that enhances nutrient uptake. The aim of this study was to evaluate the effect of AMF on yield of selected potato cultivar (Asante) under asceptic aeroponics system. Potato plantlets were inoculated with three strains of commercial AMF and control and experiment laid out in a complete randomized design. Parameters evaluated were root mycorrhizal colonization and number and weight of minitubers. *Glomus intradices* had the highest percentage of root colonization of 63% compared to 53% in *G. etunicatum*, 43% in *G. mosseae* while control had 0%. *G. intradices* increased tuber weight by 22% above the control. *G. intradices*, *G. mosseae*, *G. etunicatum* and control had 93, 84, 82 and 73 tubers per plant respectively. AMF was shown to have positive influence on production of minitubers from aeroponic system; their application can be useful in enhancing minituber production.

Key words: Aeroponics, arbuscular mycorrhiza fungi, *Glomus etunicatum*, *Solanum tuberosum*

6.2 Introduction

Potato is an important crop all over the world especially due to its high nutritive value and productivity. However, the major bottle neck to potato production low quality seeds which has resulted to use of farmer saved seeds by most farmers leading to low yields and seed degeneration (Nyende et. al., 2005). To address shortage of disease free planting materials and reduce time taken in bulking soilless-methods like aeroponics have been used to produce disease free seed potatoes

resulting to improved and quality production at reduced cost (Otazú, et. al., 2010). It has been shown to be superior to hydroponics since it results in 70% increase in tuber yield and 2.5 times more tuber number (Ritter et. al., 2001). Potatoes are suspended and grown in semi sterilized air and nutrient solution pumped to the roots system in form of mist using pumps. It allows sufficient aeration around the rhizosphere which is important for plant growth and maturity (Singh et. al., 2012). This technique has great potential as an income generator with low production cost of quality potato seeds (Chiipanthenga et. al., 2011) since it involve nutrient recirculation and has lower water and energy inputs per unit growing area (Ritter et. al., 2001; Farran et. al., 2006).

Arbuscular mycorrhiza Fungi (AMF) form symbiotic association with over 90% of plant species and have great potential in Agriculture as biofertilizers. In exchange for the photoassimilate the mycorrhiza aid the plant in nutrient absorption thereby enhancing plant growth and yield. Mycorrhizal colonization is known to take place only on young, secondary roots before suberization (Barea et. al., 1992) therefore, inoculation shortly after explants removal from culture vessels and at the beginning of root initiation Ravolanirina et. al. (1989a, 1989b) results in improved root formation and development of potato plantlets due to hormonal secretion. This improves the viability and physiological state of the potato plantlets (Hazarika and Bora, 2006). It also improves root development and growth and enhances nutrient uptake especially N, P, K (Cheng and Baumgartner, 2006). Yao et. al, (2002) observed increase in tuber number, shoot fresh weight and root dry weight in potato cultivar Goldrush inoculated with *G. etunicatum.* They further increase water uptake and protect plants from pathogenic attacks (Jeffries et. al., 2003).This results to a significant reduction chemical fertilizers and pesticide use therefore becoming a key aspect in sustainable agriculture. Aeroponic systems have also been used to develop AMF inocula (Hung et. al., 1988; Jarsfter and Sylvia 1995).

The objective of the current study was to evaluate the effect of three commercial AMF strains on minituber yield of one potato cultivar (Asante) in aeroponics system. Efficiency of mycorrhizal

association and its effect on potato will be evaluated by comparing single inoculations of three AMF strains.

6.3 Materials and methods

This experiment was established at KARI-Tigoni between October 2012 and February 2013. KARI-Tigoni station is about 40 km North-west of Nairobi city centre, with altitude of 2131m ASL, latitude of 1°15' S and longitude 23° 46' E (Jaetzold et. al., 2006). The average annual rainfall is 1096 mm with mean annual temperature between 12 and 24°C. About 280 disease free invitro Asante plantlets were obtained from Genetic Technologies Limited (GTL). Invitro propagation is known to help in preventing the transfer of major pest and diseases (Roca et. al., 1979).Three commercial Arbuscular Mycorrhizal Fungi inoculants that were tested were; *G. intradices* (AMF 1), G. *mossae* (AMF 2) and *G. etunicatum* (AMF 3) obtained from Dudutech division of Finlays Horticulture. The plantlets were hardened by growing them in trays containing sterilized sand. Each tray had 48 plantlets which were inoculated with 25g of AMF in each planting hole except for the control. The trays were placed in a shaded house (temperature range 25 – 29^0C) and watered with calcium nitrate nutrient solution when required. After 4 weeks plants had formed small stems and enough root system for transplanting in aeroponics system.

Each treatment was set in its own mini-aeroponic box measuring 18*18*27cm with three replicates giving a total of 12 mini-aeroponic boxes each containing 15 plantlets. During transplanting to the aeroponics healthy three weeks old potato seedlings were selected and secured in holes of aeroponic nutrient chamber with particular attention paid to the root system. The chambers were sprayed with mist containing calcium nitrate nutrient solution (Otazu et. al., 2010) onto the plant roots using electrical pumping system (Figure 6.1).

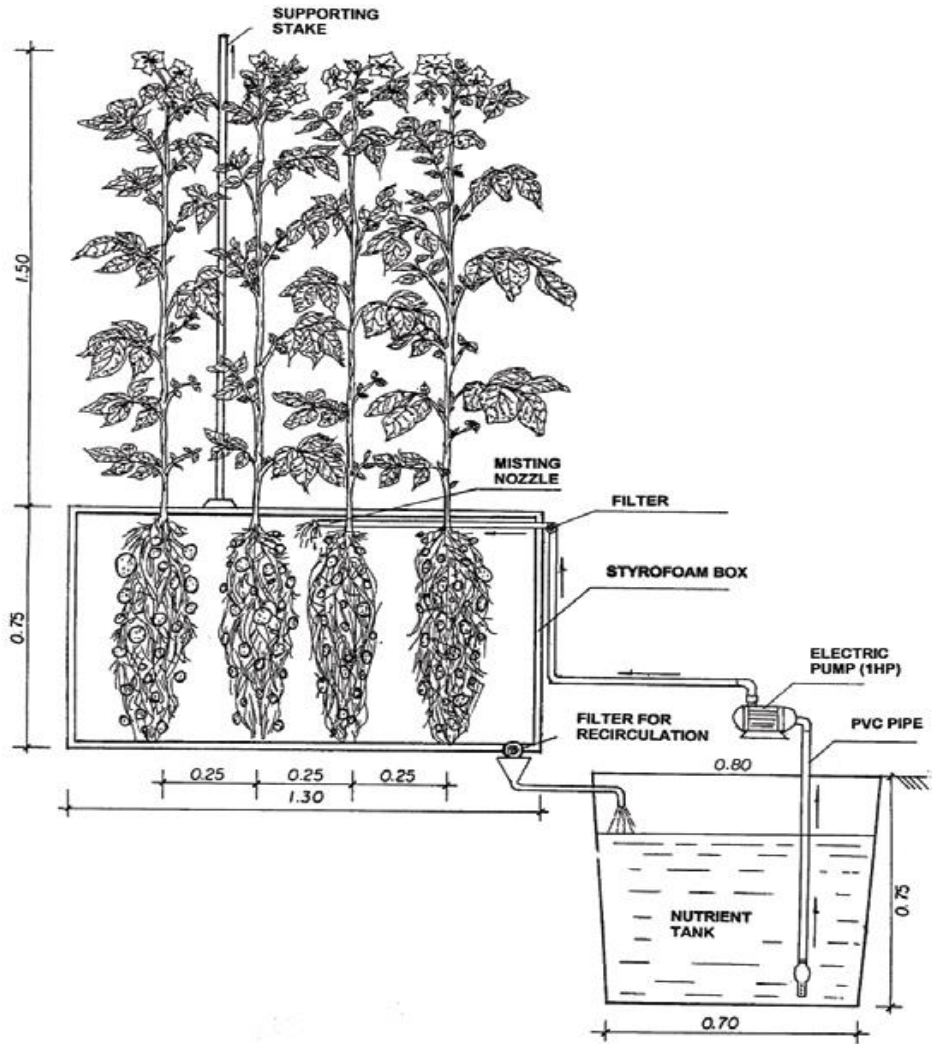


Figure 6.1: Potato growing in the aeroponic system (Source:Otazú, V. 2010).

After three weeks supporting stake was used to provide anchorage to the plants. At four weeks after transplanting, the seedlings base stems were lowered to ensure better stolon formation. At 12 weeks the first harvesting of the mini-tubers was done and their numbers recorded and graded according to size then allowed to cure in a dry and clean environment before storing in a diffused light store. Sequential harvests were done after every 10 days until the plants were five months old. Numbers of mini tubers and grades were recorded and cumulative yield of different grades calculated.

6.3.1 Data analysis

Data was subjected to one way analysis of variance using ANOVA and GENSTAT statistical package used to separate the means.

6.4 Results

6.4.1 Root colonization by AMF

There was successful root colonization in all the inoculated plants, but none for the control (Figure 6.1). *G. intradices* recorded the highest percentage of root colonization which was 1.5 times greater than in *G. etunicatum*.

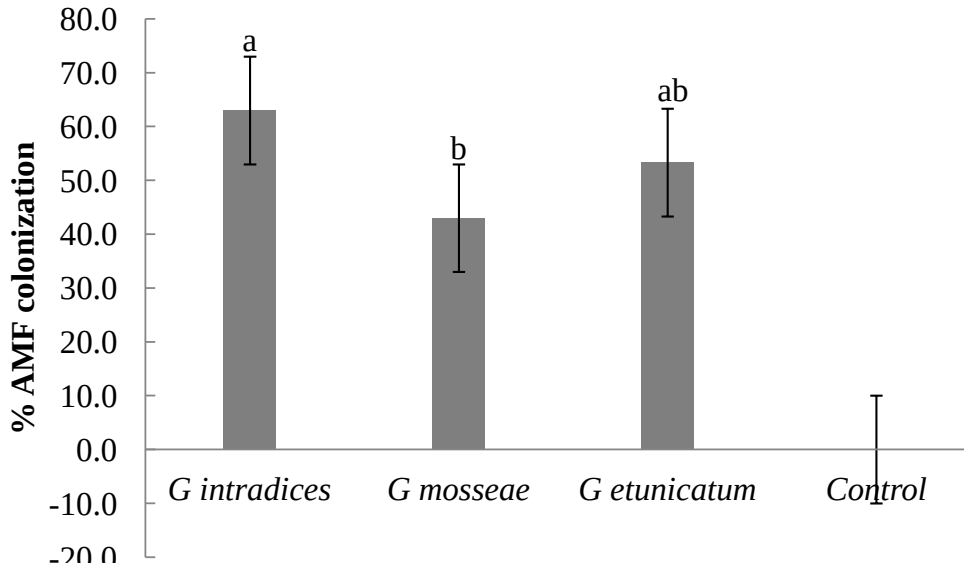
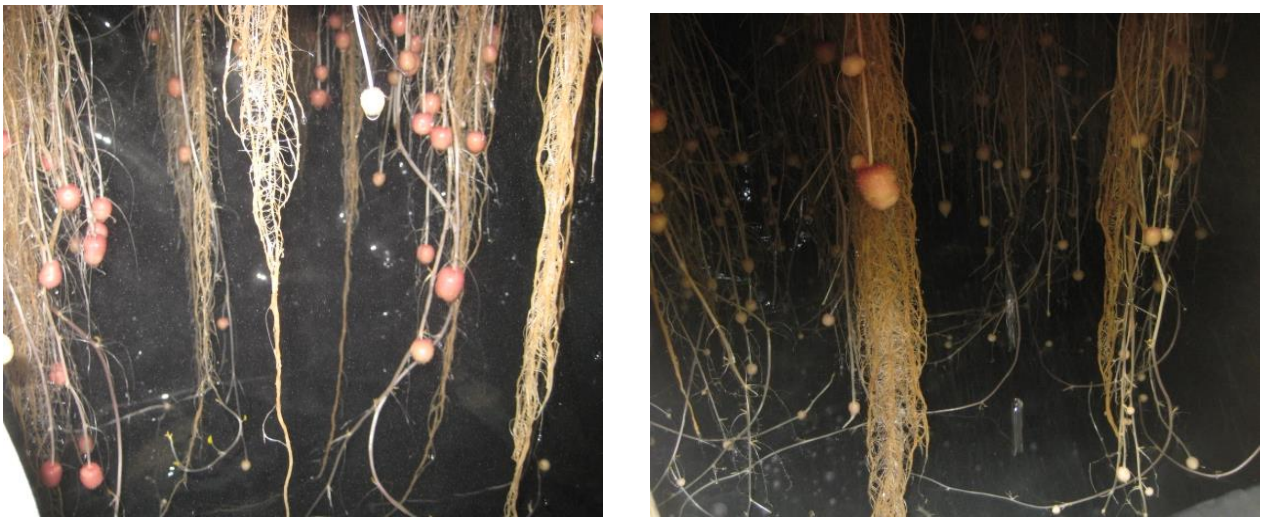


Figure 6.2: Mycorrhizal root colonization as influenced by AMF inoculum. Values with the same letter are not significantly different at P≤0.05

AMF colonization may alter root morphology by increasing the intensity of branching resulting in proliferation as shown in Plate 1.



Control ***G. mossea***

G. etunicatum ***G. intradices***

Plate 1: Effect of inoculation with three AMF species on potato roots 12 weeks after transplanting in the aeroponics system

6.4.2 Total potato tuber weight

AMF inoculation improved potato mini-tuber yield where *G. intradices* had about 0.05%, 9% and 22% more than in *G. mosseae*, *G. etunicatum* and control respectively. (Figure 6.2).

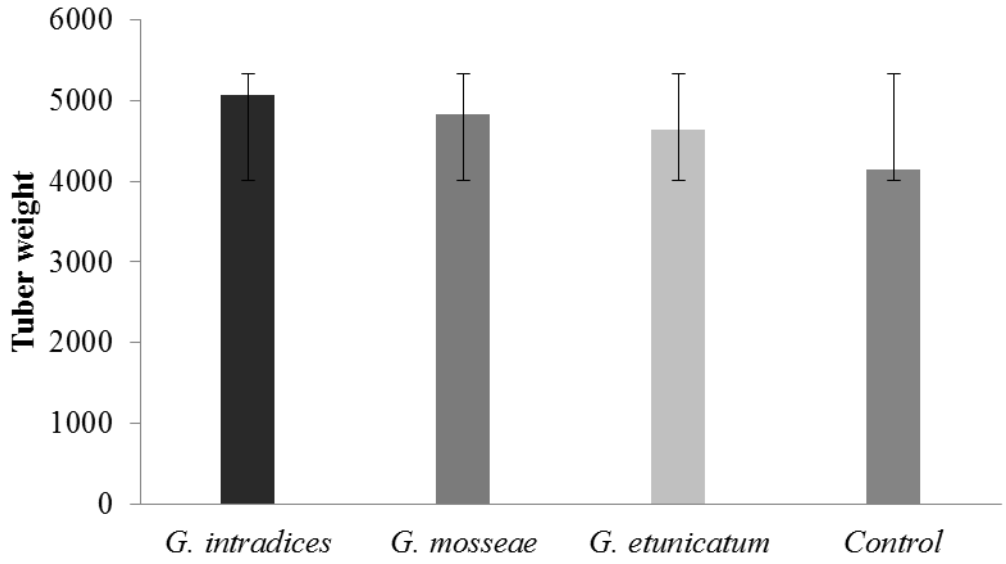


Figure 6.3: Potato tuber weight as influenced by AMF inoculation. Bars indicate standard error bars.

6.4.3 Grades on potato mini tubers as influenced by AMF inoculum

There was variation in tuber grading among the treatments (Figure 3). Each of the treatments had more small tubers followed by medium then big. The highest number of 57 big tubers was obtained with *G. etunicatum* while the lowest number of 49 big tubers was in *G.intradices* which was slightly lower than control.

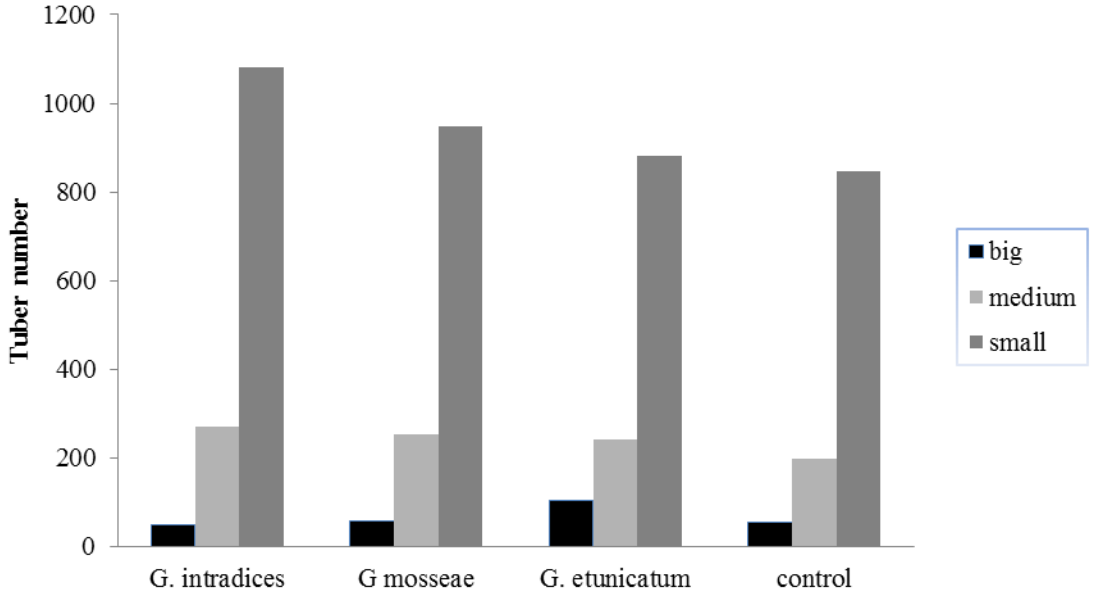


Figure 6.4: Potato tuber grades as influenced by AMF inoculates

Glomus intradices had the highest number of medium and small tubers which was 35% and 28% more compared to the uninoculated treatment. However, the same treatment had the lowest number of big tubers

6.5 Discussion

The benefit of mycorrhiza depends on its ability to colonize the roots and establish a symbiotic association with the host plant (Roberto et. al., 2012). AMF colonization in all the inoculated treatments shows the compatibility of mycorrhiza with potato. The effect of AMF has been shown to vary in the interaction with their hosts, and this variation has been observed even at the plant cultivar level (Estaún et. al. 2010). Differences in root colonization among the three AMF strains shows that plants do exhibit some form of specificity or preference for individual AMF resulting to different levels of mycorrhizal root colonization (Smith et. al., 2009). AMF have also been shown to be able to alter the root morphology Saraswati et. al., (2012) leading to increased surface area for water and nutrient absorption.

Increase in tuber weight in the AMF inoculated treatments compared to control may be due to an increased root surface area by the extraradical mycelium of the AMF which provide more root volume for nutrient absorption (Vosátka et. al., 2000). Nutrients absorption is said to be faster in AMF colonized plants compared to the uncolonized plants (Duffy et. al., 1999). There were also different responses to the host plant due to the various AMF inoculants (Munkvold et. al., 2004). The bio-effectiveness of AMF on host plant varied depending on the species of AMF (Vierheilig et. al., 2008).

Glomus intradices had the highest number of medium and small tubers but the lowest number of big tubers. This is contrary to what is expected of a mature potato crop that provides higher tuber yields with larger tubers (Duffy et. al., 2000) as was in *Glomus etunicatum.* This may be attributed to environmental factors like temperature affecting tuber yield as it influences tuber enlargement (Van

Ittersum and Scholte, 1992, Tsako et. al., 2012). Aeroponic systems are known to produce more tubers than conventional method of planting in substrate because of the sequential harvesting due to the breaking of apical dormancy thereby promoting further tuberization hence yield increase (Otazu, 2007). Furthermore in the aeroponic chamber there is adequate aeration with no mechanical obstructions for root development. Size of the minituber is an important quality aspect as it determines the number of eyes, sprouts and stem per tuber which is further influenced by temperature (Struik and Wiersema, 1999). Therefore the best inoculum that produced quality minitubers was in *Glomus etunicatum.* Combining micro-propagation and AMF inoculation increased the potato seed yield and quality of the minitubers. Furthermore, *Glomus etunicatum* had the lowest root colonization signifying that the extent of root colonization did not correlate with the benefits conferred to the host plant. Stoner and Clawson (1998) reported that over 70 tubers per plant can be achieved under aeroponics. Plants inoculated with the three AMF strains namely; *G. intradices, G. mosseae, G. etunicatum* and control produced 93, 84, 82 and 73 tubers per plant, respectively. Improved tuber yield and grades are attributed to mycorrhizal inoculants as a result of improved nutrient uptake and should be enhanced to increase production.

6.6 Conclusions

The results indicate that potato responded well to AMF inoculation. Repeated harvesting in the aeroponics offers the possibility of obtaining high number of tubers and this was further enhanced with AMF inoculation especially *G. etunicatum*. Use of AMF inoculants can therefore increase the availability of healthy clean potato seeds.

CHAPTER SEVEN

7.1 CONCLUSIONS

1. Microbial inoculants namely; Arbuscular Mycorrhizal Fungi (AMF) and rhizobacteria were effective biofertilizers of potato as they were able to enhance tuber yield and P nutrient content of the shoot.

2. Multiple inoculants with several microbial isolates; *G. intradices* + *Pseudomonas*, *G. etunicatum* + *Bacillus*, *G. intradices* + *Bacillus* and *Pseudomonas* + *Azoctobacter* + *Bacillus* showed synergistic effects on suppression of bacterial wilt.

3. The commercial AMF especially *G. intradices* were effective root colonizers of potato (Asante variety) under the aeroponic system and increased root proliferation hence high tuber number.

7.2 RECOMMENDATIONS

i) Considering the immense importance of potato and the threat caused by Bacterial wilt; besides the use of resistant varieties and organic amendments, biocontrol methods can also be an option for disease control and growth enhancement with respect to being ecofriendly, non-hazardous and non-toxic reducing the cost of potato production.

ii) Dual inoculation of potato with rhizobacteria and AMF and combining multiple strains of rhizobacteria is recommended as it significantly enhances yield and control bacterial wilt which must be seed coated or inoculated around the roots of the plants to ensure effectiveness of the inoculants. However, despite there being no disease symptoms on the plants, latent infection test of potato seeds is critical to ensure disease free planting materials so as to control the bacterial wilt menace.

iii) Further research on the mode, timing and amount of the inoculant application especially in extensive field research trials under taken under various soils and farm environment to determine the performance of these bio-inoculants.

8.0 SUMMARY

Potato is an important food crop in Kenya due to the critical role it plays of ensuring food security and nutrition therefore alleviating hunger. Despite efforts made to enhance its production low productivity still remain a major constrain. This is mainly due to reduced soil fertility and high incidences of diseases especially bacterial wilt. In this thesis, we proposed to examine the effectiveness of beneficial microorganisms namely; Arbuscular mycorrhiza fungi (AMF) and rhizobacteria in enhancing nutrient uptake and management of Bacterial wilt disease as key to improving crop productivity.

The methodological approach used in this study was under controlled conditions and involved assessment of:

1. Evaluation of Arbuscular Mycorrhizal Fungi (AMF) and rhizobacteria inoculants on performance of potato
2. Performance of rhizobacteria and AMF inoculants in control of Bacterial wilt in Potato production
3. Performance of potato inoculated with Arbuscular Mycorrhizal Fungi (AMF) under Aeroponic System

In chapter 3, we evaluated AMF and rhizobacteria inoculants on performance of potato. This study was conducted using three potato cultivars with three commercial AMF and *Pseudomonas* as the microbial inoculants in single and duo inoculations. Combination of an AMF and *Pseudomonas* were considered effective in improving the yield of potato and enhancing shoot Phosphorus content. Presence of indigenous AMF spores showed that soil solarization did not destroy the beneficial microorganisms.

In chapter 4 and 5, effectiveness of three AMF and rhizobacteria inoculants on two potato cultivars; Tigoni and clone (387164.4) were evaluated in control of bacterial wilt in two experiments. The studies showed that combined inoculants and organic amendments especially poultry manure were

effective in controlling bacterial despite the low potato yield especially in Tigoni, the susceptible cultivar. Latent tuber infection test was shown to be critical even in plants that are appear symptomless which may otherwise test positive as in the case of *G. etunicatum* and *Bacillus* in Tigoni.

In chapter 6, Performance of potato inoculated with three AMF was evaluated under Aeroponic System. The study showed increase in potato tuber weight and number in the inoculated compared to control due to root proliferation and AMF colonization. There is urgent need to further test these microorganisms especially in under field conditions to determine their effectiveness and efficiency in improving crop productivity.

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10.0: APPENDICES

Appendix 1: Modified SMSA for isolation of *Ralstonia solanacearum* (As used at KARI-NARL, Plant Pathology Section)

Preparation of the basal medium

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

Preparation of additive solutions (filter-sterilized) and incorporation into basal medium

Additive

Additive	**stock solution concentration**	**required concentration in basal medium**	**Amount to add to 250ml basal medium**
Crystal violet	0.1g in 10mls water	5mg/litre	125µl
Chloramphenicol	0.1g in 10mls Methanol	5mg/litre	125µl
Penicillin G	0.01g in 10mls water	0.5mg/litre	125µl
Bacitracin	0.1g in 10mls Methanol	25mg/litre	625µl
Tetrazolium salts	0.1g in 10mls water	50mg/litre	1250µl
Polymixin B Sulphate	0.1g in 10mls water	100mg/litre	2500µl

Appendix 2: CPG Medium for cultivation of *Ralstonia solanacearum*

Casamino acids (Difco) 1.0g

Bacto-Peptone (Difco) 10.0g

Glucose 10.0g

Bacto-Agar (Difco) 18.0g

Distilled water 1000ml

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

ANOVA TABLES

Appendix 3: Pot experiment

Big tubers refer to figure 3.1

Source	DF	Type III SS	Mean Square	F Value	Pr > F
variety	2	13.38888889	6.69444444	4.83	0.0106
block	3	17.81481481	5.9382716	4.28	0.0075
trt	8	34.33333333	4.29166667	3.09	0.0044
variety*trt	16	25.27777778	1.57986111	1.14	0.3359

Medium tubers

Source	DF	Type III SS	Mean Square	F Value	Pr > F
variety	2	124.0185185	62.0092593	17.48	<.0001
block	3	23.2962963	7.7654321	2.19	0.096
trt	8	60.2407407	7.5300926	2.12	0.0433
variety*trt	16	61.4814815	3.8425926	1.08	0.3852

Small tubers refer to figure 3.1

Source	DF	Anova SS	Mean Square	F Value	Pr > F
variety	2	188.35185	94.1759259	24.81	<.0001
block	3	20.694444	6.8981481	1.82	0.1509
trt	8	95.351852	11.9189815	3.14	0.0039
variety*trt	16	42.981482	2.6863426	0.71	0.7779

Tuber weight refer to figure 3.2

Source	DF	Anova SS	Mean Square	F Value	Pr > F
variety	2	281.46296	140.73148	1.64	0.2011
block	3	1661.5833	553.86111	6.44	0.0006
trt	8	17649.352	2206.16898	25.67	<.0001
variety*trt	16	734.87037	45.9294	0.53	0.9206

Shoot biomass refer to table 3.2

Source	DF	Anova SS	Mean Square	F Value	Pr > F
variety	2	0.4612911	0.23064557	47.75	<.0001
block	3	0.135624	0.045208	9.36	<.0001
trt	8	2.7291539	0.34114423	70.62	<.0001
variety*trt	16	0.1629646	0.01018529	2.11	0.0159

Phosphorus refer to table 3.3

Source	DF	Anova SS	Mean Square	F Value	Pr > F
variety	2	0.0012056	0.00060278	3.97	0.0228
block	3	0.002188	0.00072932	4.81	0.004
trt	8	0.012	0.0015	9.88	<.0001
variety*trt	16	0.0052611	0.00032882	2.17	0.0129

% Mycorrhizal root colonization refer to table 3.4

Source	DF	Anova SS	Mean Square	F Value	Pr > F
variety	2	1017.1852	508.592593	21.7	<.0001
block	3	277.80556	92.601852	3.95	0.0112
trt	8	4723.8519	590.481481	25.2	<.0001
variety*trt	16	767.31482	47.957176	2.05	0.0198

Inoculated spore count refer to figure 3.3

Source	DF	Anova SS	Mean Square	F Value	Pr > F
variety	2	91.583333	45.791667	0.38	0.6887
block	3	802.04167	267.347222	2.19	0.1001
trt	5	2049.9583	409.991667	3.36	0.0106
variety*trt	10	519.58333	51.958333	0.43	0.9271

Indigenous spore count refer to figure 3.4

Source	DF	Anova SS	Mean Square	F Value	Pr > F
variety	2	0.0154152	0.00770762	0.87	0.4218
block	3	0.1899106	0.06330353	7.17	0.0003
trt	8	1.127825	0.14097813	15.97	<.0001
variety*trt	16	0.1413183	0.0088324	1	0.4655

APPENDIX 4: AMF and rhizobacteria experiment

AUDPC refer to figure 4.1

Source	DF	Type III SS	Mean Square	F Value	Pr > F
variety	1	5599346.48	5599346.48	180.47	<.0001
rep	3	135064.45	45021.48	1.45	0.2341
trt	13	87791519.9	6753193.84	217.66	<.0001
variety*trt	13	1766769.92	135905.38	4.38	<.0001

Medium tubers refer to figure 4.2

Source	DF	Anova SS	Mean Square	F Value	Pr > F
variety	1	322.321429	322.3214286	51.42	<.0001
block	3	16.7857143	5.5952381	0.89	0.4487
Trt	13	203.464286	15.6510989	2.5	0.0064
variety*Trt	13	81.4285714	6.2637363	1	0.4599

Small tubers

Source	DF	Anova SS	Mean Square	F Value	Pr > F
variety	1	3.9375	3.9375	0.34	0.5609
block	3	54.9553571	18.3184524	1.59	0.199
Trt	13	155.401786	11.9539835	1.04	0.427
variety*Trt	13	114.6875	8.8221154	0.76	0.6948

Tuber weight refer to figure 4.3

Source	DF	Anova SS	Mean Square	F Value	Pr > F
variety	1	190492.509	190492.5089	22.4	<.0001
block	3	35078.5982	11692.8661	1.38	0.2562
Trt	13	415824.688	31986.5144	3.76	0.0001
variety*Trt	13	108638.616	8356.8166	0.98	0.4753

Mycorrhizal root colonization refer to table 4.1

Source	DF	Anova SS	Mean Square	F Value	Pr > F
variety	1	167.58036	167.58036	20.38	<.0001
block	3	95.24107	31.74702	3.86	0.0123
Trt	13	21785.4018	1675.80014	203.81	<.0001
variety*Trt	13	427.04464	32.84959	4	<.0001

Refer to table 4.3 for tuber mineral content

N

Source	DF	Anova SS	Mean Square	F Value	Pr > F
variety	1	2.64045714	2.64045714	12.49	0.0015
block	1	0.38777857	0.38777857	1.83	0.1869
Trt	13	4.38589286	0.33737637	1.6	0.1482
variety*Trt	13	1.03084286	0.0792956	0.37	0.9668

P

Source	DF	Anova SS	Mean Square	F Value	Pr > F
variety	1	0.04017857	0.04017857	18.91	0.0002
block	1	0.00002857	0.00002857	0.01	0.9085
Trt	13	0.02454286	0.00188791	0.89	0.574
variety*Trt	13	0.02062143	0.00158626	0.75	0.7044

K

Source	DF	Anova SS	Mean Square	F Value	Pr > F
variety	1	1.82521607	1.82521607	22.2	<.0001
block	1	0.28714464	0.28714464	3.49	0.0725
Trt	13	1.53678036	0.11821387	1.44	0.2058
variety*Trt	13	0.54520893	0.04193915	0.51	0.8986

Mn

Source	DF	Anova SS	Mean Square	F Value	Pr > F
variety	1	20.1480018	20.1480018	2.22	0.1475
block	1	42.8575018	42.8575018	4.73	0.0386
Trt	13	161.9368589	12.4566815	1.37	0.2342
variety*Trt	13	69.8191732	5.3707056	0.59	0.8386

B

Source	DF	Anova SS	Mean Square	F Value	Pr > F
variety	1	3.0973018	3.0973018	0.33	0.571
block	1	2.6622161	2.6622161	0.28	0.5992
Trt	13	115.8274732	8.9098056	0.95	0.5228
variety*Trt	13	32.5867732	2.5066749	0.27	0.9923

Zn

Source	DF	Anova SS	Mean Square	F Value	Pr > F
variety	1	212.1607143	212.160714	3.67	0.0659
block	1	118.9028571	118.902857	2.06	0.1628
Trt	13	765.0971429	58.8536264	1.02	0.4622
variety*Trt	13	629.4492857	48.4191758	0.84	0.6197

Fe

Source	DF	Anova SS	Mean Square	F Value	Pr > F
variety	1	6851.00643	6851.00643	5.52	0.0263
block	1	233.70286	233.70286	0.19	0.6677
Trt	13	25386.24	1952.78769	1.57	0.1547
variety*Trt	13	8614.31357	662.63951	0.53	0.8822

Cu

Source	DF	Anova SS	Mean Square	F Value	Pr > F
variety	1	101.2785018	101.278502	36.49	<.0001
block	1	5.9085018	5.9085018	2.13	0.1561
Trt	13	68.8137304	5.2933639	1.91	0.0762
variety*Trt	13	29.1344732	2.2411133	0.81	0.6481

APPENDIX 5: Biocontrol experiment

AUDPC refer to figure 5.1

Source	DF	Anova SS	Mean Square	F Value	Pr > F
rep	3	2572.74337	857.58112	20.41	<.0001
trt	10	10045.8528	1004.58528	23.91	<.0001
variety*trt	10	636.98273	63.69827	1.52	0.1546

Tuber weight refer to figure 5.2

Source	DF	Anova SS	Mean Square	F Value	Pr > F
variety	1	8940.557	8940.557	0.52	0.4722
block	3	1453014.13	484338.042	28.34	<.0001
trt	10	417849.114	41784.911	2.44	0.0155
variety*trt	10	97299.068	9729.907	0.57	0.8327

Medium tubers

Source	DF	Anova SS	Mean Square	F Value	Pr > F
variety	1	6.5454545	6.5454545	0.86	0.3571
block	3	167.772727	55.9242424	7.35	0.0003
trt	10	143	14.3	1.88	0.0648
variety*trt	10	41.9545455	4.1954545	0.55	0.8465

Small tubers

Source	DF	Anova SS	Mean Square	F Value	Pr > F
variety	1	19.1022727	19.1022727	1.92	0.1707
block	3	204.488636	68.1628788	6.85	0.0005
trt	10	110.090909	11.0090909	1.11	0.3714
variety*trt	10	42.2727273	4.2272727	0.42	0.9292

Phosphorus

Source	DF	Anova SS	Mean Square	F Value	Pr > F
variety	1	0.00100227	0.00100227	0.44	0.5157
block	1	0.00791136	0.00791136	3.45	0.0773
trt	10	0.04376364	0.00437636	1.91	0.1019
variety*trt	10	0.01637273	0.00163727	0.71	0.7023