BIOCONTROL OF LATE BLIGHT *Phytophthora infestans* ON POTATO USING SELECTED FUNGAL ANTAGONISTS AND PLANT EXTRACTS IN KIAMBU AND NYANDARUA COUNTIES, KENYA

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DECLARATION

I Steve Ochieng Agong declare that this thesis is my original work and has not been presented for the award of a degree in any other university or any other award.

Signature.. Date 7/10/2021..................................

Department of Agricultural Science and Technology.

University supervisors' approval

We confirm that the work reported in this thesis was carried out by the candidate under our supervision and has been submitted with our approval as university supervisors.

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DEDICATION

This thesis is in a special way dedicated to my parents Mrs. Margaret Omolo Symon and Mr. Simon Yasson Agong. It is also dedicated to my siblings; Ronnie, Brian, Curtis and Nicole along with my extended family and friends.

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LIST OF ABBREVIATIONS AND ACRONYMS

%Y/C	Percentage Yield Over Control
ADC	Agricultural Development Corporation
AGRA	Alliance for a Green Revolution in Africa
ANN	Africa News Network
ANOVA	Analysis of Variance
ASDSP	Agricultural Sector Development Support Program
AUDPC	Area Under the Disease Progress Curve
BCA	Biological Control Agent
CFU	Colony Forming Units
CIDP	County Integrated Development Plan
CIP	International Potato Center
CMC	Carboxy-methyl cellulose
DAI	Days after Inoculation
DI	Disease Index
FAO	Food and Agriculture Organization of the United Nations
GAPs	Good Agricultural Practices
GPS	Global Positioning System

IDM	Integrated Disease Management
KALRO	Kenya Agriculture and Livestock Research Organization
KU	Kenyatta University
LSD	Least Significant Difference
NARL	National Agricultural Research Laboratories
NPCK	National Potato Council of Kenya
PCPB	Pest Control Products Board
PDI	Percentage Disease Index
PGI	Percentage Growth Inhibition
PGS	Percentage Growth Suppression
PI	Percentage Incidence/Infection
SAR	Systemic Acquired Resistance
SAS	Statistical Analysis Software
SPSS	Statistical Package for Social Sciences

ABSTRACT

Potato late blight (*Phytophthora infestans* (Mont.) de Bary) is a major threat to potato production instigating overreliance on synthetic fungicides. Synthetics however, cause human health complications, environmental pollution and resistance development by late blight; this can be substituted with safer biological control options. The study objective was to enhance potato production through sustainable management of potato late blight. In May 2018, a baseline survey was carried out in Nyandarua County to assess the socio-demographic factors, potato production and crop management practices that impact on prevalence of potato late blight. Overall, 105 small scale farmers were interviewed using semi-structured questionnaires. Survey data was analyzed using SPSS v.20. Fungi were isolated from potato rhizosphere using serial dilution method and subsequently screened *in vitro* via dual culture technique. Crude extracts were prepared from bioactive plants using maceration technique then evaluated *in vitro* via poison food technique. Data, on *P. infestans* mycelial growth inhibition and suppression by fungal isolates and crude extracts, respectively, was analyzed using SAS v.9.2 at $P \leq 0.05$. *Azadirachta indica* extract and *Trichoderma hamatum* isolate caused significant suppressive and inhibitory effects *in vitro* of up to 59.1 and 50.2%, respectively. *Trichoderma harzianum, T. afroharzianum, T. asperellum*, *A. indica*, *Pistacia lentiscus* and *Tithonia diversifolia* significantly ($P<0.05$) suppressed pathogen growth *in vitro* thus were considered for further screening in the field. Isolates were tested on potato cultivars, Shangi and Tigoni, compared to water and Master® as controls. The trial was an RCBD for three cropping seasons, including two long rains (May to August 2018 and April to July 2019) and one short rain (December 2018 to March 2019). Data on disease incidence, severity and yield was analyzed using SAS v.9.2 at $P \leq 0.05$. Findings showed male dominance in potato production at 62%, 43.8% of respondents attained primary education, 74.3% used saved seeds and 65.8% used a single fungicide class. Under field evaluation, *T. hamatum* significantly ($P<0.001$) reduced disease incidence and severity on Shangi and Tigoni by up to 92.7±4.3 and 54±6.1%, respectively (May to August 2018). *Azadirachta indica* and *T. hamatum* supressed disease up to 89.1±2.3 and 65.2±4.5%, respectively, on Tigoni variety. Fungal antagonists generally caused significantly ($P \leq 0.001$) higher yields compared to plant extracts. During May to August 2018 and April to July 2019, *T. harzianum* and *T. diversifolia* yielded up to 3.65±1.2t/ha and 5.75±1.3t/ha, respectively. Education is considered important as knowledge level influences decision making and adoption of technologies and practices. Use of farm saved seeds is a potential source of inoculum and use of single fungicide class could lead to resistance development. Therefore, enhancing farmers' knowledge and promoting good agricultural practices that include IPM can improve management of potato late blight thus enhancing productivity. These results demonstrate existence of biologically active microorganisms and crude extracts from plants in the local environment that can be exploited towards developing affordable and safer management products for use against *P. infestans.*

CHAPTER ONE: INTRODUCTION

1.1 Background information

Potato (*Solanum tuberosum* L.) is a member of the Solanaceae family that was first domesticated in Peru, South Asia over 1,000 years ago (Levy and Rabinowitch, 2017). The crop has since then been distributed all over the world where it now ranks fourth most important food crop, after maize, wheat and rice (Islam *et al.,* 2018). Its total global production, as at 2019, is estimated at about 370.43 million metric tons consumed by more than one billion people (CIP, 2019; Statista, 2021). Kenya is the fifth biggest potato producer in Africa (Taiy *et al.,* 2017) realizing annual yields of up to 2 million tonnes annually (FAOSTAT, 2019) and on average 8 – 15 t/ha which is about three times lower than the 30 – 40 t/ha potential (Gitari *et al.*, 2018a and b).

Potato remains the second most important staple food crop in Kenya, after maize, and contributes up to Ksh 50 billion annually (Muthoni *et al.*, 2017; Mumia *et al.,* 2018). The potato consumption per capita annually in Kenya is 30kg which is expected to increase with the growth of the fast-food industry. About 800,000 farmers grow potatoes in Kenya out of which approximately 90% are small scale (Muthoni *et al.*, 2017; Mutegi *et al.*, 2021). The potato subsector employs close to 3.3 million people along the value chain as market agents, transporters, processors, vendors and exporters (AGRA, 2019).

Potato production is faced by a number of challenges which include inadequate disease-free planting materials, pests and diseases (Riungu, 2011; Muthoni *et al.,* 2013; Karanja *et al.,* 2014). The two major diseases affecting the potatoes include bacterial wilt by *Ralstonia solanacearum* (Smith) and late blight by

Phytophthora infestans (Mont.) de Bary (Karanja *et al.,* 2014), where potato late blight is second in prevalence after bacterial wilt with over 67% (Kaguongo *et al.,* 2010).

Phytophthora infestans is one of the world's most damaging plant diseases. It is documented to have caused the Great Irish Famine after it destroyed potato crops in Europe from 1845 to 1847 leading to mass starvation and death of up to one million people, forcing over one million people to migrate to the rest of Europe and USA (Ray *et al.,* 2018). According to Nyankanga *et al.* (2004), approximately 30-60% of potato crop is lost to late blight annually in Kenya. In agreement with previous reports, Miriita *et al.* (2016) and Kisinga (2019) state that, in Kenya, the pathogen causes 30 to 75% yield losses, even up to 100% in susceptible cultivars.

Majority of potato farmers mainly rely on synthetic chemicals to manage potato diseases (Yanar *et al.,* 2011). This overreliance is because there exist fewer alternative management options rather than chemical fungicides (Tsedaley, 2014) since chemical management strategy is the major approach utilized globally to contain late blight (Tsedaley, 2014). Additionally, famers know not of any other management methods (Nyakanga *et al.,* 2004) and also due to the quick action of synthetic chemicals (Anju *et al.,* 2017).

Excessive misuse of synthetic pesticides, on the other hand, has over time led to accumulation of pesticide residues (Carvalho, 2017), environmental pollution, resistance development, and health hazards to humans (Zaker, 2016). An alternative to synthetic chemicals in plant disease management, is biological control, which has attracted a lot of attention in the recent years (Marutescu *et al.,* 2017). Biological control strategy is environment friendly and has greater public acceptance compared to synthetic pesticides. It has less adverse effect on non-target organisms and less likelihood of resistance developing (Zaker, 2016). Thus, fits perfectly in the Integrated Disease Management (IDM) concept (Tabassum and Vidyasagar, 2013; Zaker, 2016)

1.2 Problem statement

Late blight disease remains a major global threat to food security (Hu *et al.*, 2012; Fry *et al.*, 2015). Global conservative potato yield loss estimates are over $6 billion, per year with fungicides alone accounting for over $1 billion (Hussain *et al.*, 2015). In Kenya, potato late blight is estimated to affect two-thirds of all potato farms (D'Alessandro *et al.*, 2015). There have been several fungicides recommended against this pathogen (Yao *et al.*, 2016) but they only offer short-term solutions, are costly, risky to health and cause other health and environmental hazards (Riungu, 2011). Furthermore, most fungicides are protective with no curative effect (Binyam, 2014).

There has been an increase in use of fungicides to manage late blight disease, which has led to new and more aggressive biotypes of *P. infestans* emerging (Mekonen and Tadesse, 2018). There is therefore need for alternative control methods for late blight. Although there is an increase in the number of bio control products for plant diseases, fungicides still account for 15% of total chemicals used in agriculture while bio control products represent only 1% (Junaid *et al.*, 2013). At the moment, there is no registered biopesticide specifically for the management of late blight on potatoes in Kenya. BIO CURE F 1.5 WP (*Trichoderma viride* strain TV-1) 1.5×10^6cfu/g is the only biopesticide registered for broad spectrum management of soil-borne fungal pathogens including the *Phytophthora* sp., but only on roses (PCPB 2021).

Additionally, not much work has been done on biological control of potato late blight in Kenya. Therefore, there is need to increase bioprospecting to identify effective biocontrol agents that are safer and reduce the use of chemical fungicides.

1.3 Justification of the study

All countries have a major objective of increasing food productivity to address the issue of food and nutrition security. The Food and Agriculture Organization (FAO) of the United Nations has predicted the need for a 70% increase in food production in the world so as to balance the food demand of the fast-growing population (Sasson, 2012).

In Kenya, potato is second after maize in importance of food and nutrition security and contributes up to 50 billion Kenya shillings annually (Muthoni *et al.,* 2017; Mumia *et al.,* 2018). The potato value chain further employs over 2 million people at various levels, contributing to house hold income (CIP, 2019). Globally, it is estimated that pathogenic microorganisms and pests cause loss of up to 36.5% of total yield in potatoes (Ons *et al.*, 2020)

Potato late blight is a major disease negatively impacting potato production, with a prevalence of over 67% in Kenya (Kaguongo *et al.,* 2010; Karanja *et al.,* 2014). In Kenya, potato late blight is responsible for annual losses of approximately 30 to 75% crop and yield losses (Nyankanga *et al.,* 2004; Miriita *et al.,* 2016) and can lead to up to 100% losses on susceptible cultivars if not controlled (Miriita *et al.*, 2016; Kisinga, 2019)

Potato late blight has put a lot of pressure on farmers forcing them to rely heavily on synthetic pesticides to mitigate the effects of the disease (Rani *et al.,* 2017). About 93 to 100% of potato farmers in Kenya solely rely on synthetic pesticides to control late blight (Gianessi and Williams, 2011). The sole overreliance on synthetic chemicals very often has led to several acute and chronic human illnesses. These illnesses that have been associated with exposure to chemical pesticides, which alter the food chain and bio-accumulate in the higher tropic level (Mostafalou and Abdollahi, 2012; Begum and Rajesh, 2015). Pesticides have overtime led to resistance development by the pathogen (Zaker, 2016).

There is a rising global shift towards reducing the application of chemical fungicides on agricultural produce and therefore, there is a strong public and scientific desire to come up with alternatives to the chemical fungicides guaranteeing safety to humans, the environment and non-target organisms with economically friendly benefits (Mari *et al.,* 2007; Lal *et al.,* 2016), an alternative which biological control offers with the advantages of reduced health and environmental impacts in addition to greater public acceptance (Reino *et al.,* 2008; Begum and Rajesh, 2015; Lal *et al.*, 2016).

Potato is best cultivated at an altitude of between 1,500 and 3,500 meters above sea level (masl) (Janssens *et al.,* 2013; Muthoni and Kabira, 2015; Mutegi *et al.*, 2021). The study was carried out in Kiambu and Nyandarua Counties which lie within the recommended potato growing altitudes (Jaetzold *et al*., 2007; Janssens *et al.,* 2013; Mutegi *et al.,* 2021). Additionally, These Counties are among the major potato producers in central region of Kenya where there have been several previous reports of late blight (Were *et al.,* 2013; Kaguongo *et al.,* 2010). The technologies under evaluation can thus be replicated in both Counties and elsewhere (Mwangi, personal communication, August 12, 2021).

1.4 Research objectives

1.4.1 Broad objective

To enhance potato production through sustainable management of potato late blight.

1.4.2 Specific objectives

i. To assess the socio-demographic factors, potato production and crop management practices that impact on prevalence of late blight on potato in Nyandarua County.

ii. To isolate fungi from potato rhizosphere and evaluate their antagonistic potential against *Phytophthora infestans in vitro* and under field conditions in Kiambu County.

iii. To identify plants with bioactive potential against *Phytophthora infestans in vitro* and under field conditions in Kiambu County.

1.5 Hypotheses

i. Socio-demographic factors, crop production and disease management practices significantly affect the prevalence of late blight on potato in Nyandarua County.

ii. Fungal isolates from potato phyllosphere and rhizosphere effectively suppress *Phytophthora infestans in vitro* and under field conditions.

iii. Bioactive plant extracts effectively suppress *Phytophthora infestans in vitro* and under field conditions.

1.6 Significance of the study

This study aimed to scout for antagonistic microbes and plants with suppressive potential from the local environment and evaluate their bioactive activity against potato late blight disease both *in vitro* and *in vivo.* This being a component of biological control, it will fit well into integrated disease management strategy (IDM). Championing for biological control will encourage reduction in the use of synthetics in the management of potato late blight and other diseases. The findings from this study shall enlighten local manufacturers of plant protection products on the rich biodiversity of microbes and plants in local environments, that can be exploited to develop alternative biological control products that are affordable and safer.

1.7 Conceptual framework

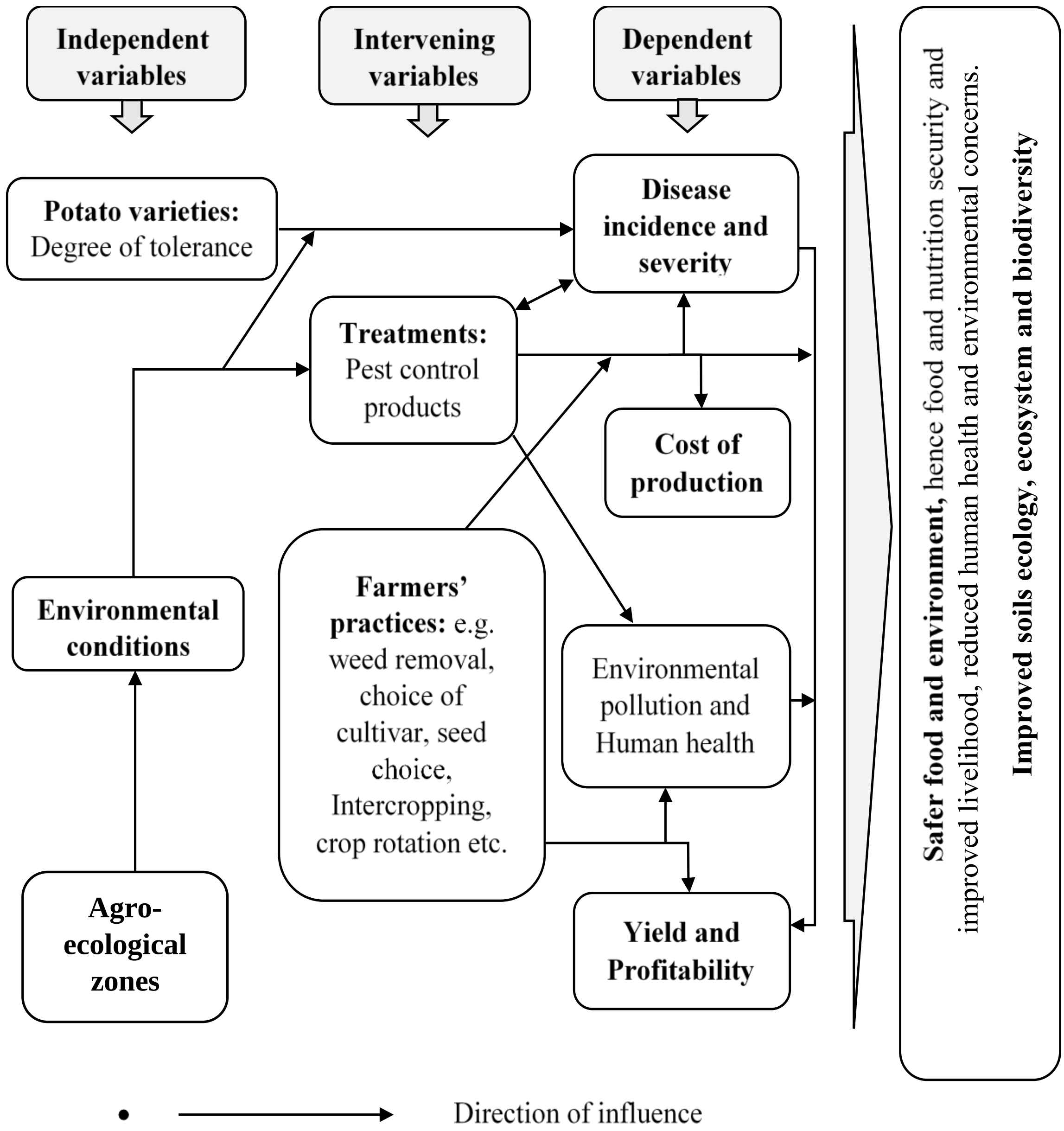


Figure 1.1 Conceptual framework

CHAPTER TWO: LITERATURE REVIEW

2.1 Importance of potato production in Kenya

2.1.1 Economic importance

As a food security crop and cash crop, potato is economically and nutritionally important in developing countries (Walker *et al.*, 2011). Economically, it's an important cash crop which provides ready cash to farmers since it has a short maturity period of between 3 to 4 months and a high market value. Potato employs about 3.3 million people along its value chain generating income of approximately USD 480 million annually (Parker, 2021). Other than for human consumption, potato can be used in industrial processing (i.e., starch, glue, and fuel-grade ethanol in distilling industry) and for animal feed stuff fermented in silage (Zarzecka, 2009; Ortiz and Mares, 2017).

Starch derived from potato is widely used by pharmaceutical, textile, wood, and paper industries as an adhesive, binder, texture agent, and filler, and by oil drilling firms to wash boreholes. Starch from is a 100% biodegradable substitute for polystyrene and other plastics also used, for instance, in disposable plates, dishes, and knives. Potato peels and other wastes from potato processing are rich in starch that can be liquefied and fermented to produce fuel-grade ethanol (Ortiz and Mares, 2017; CIP 2021).

2.1.2 Nutritional importance

The total global potato production is about 400 million tonnes annually (Zhao *et al*., 2020). Potato ranks fourth most important food crop after wheat *Triticum aestivum* L., rice *Oryza sativa* L. and maize *Zea mays* L., respectively, in the world (Islam *et al.*, 2018). In Kenya, potato is one of the important food crops, second in rank after maize in terms of production (Mumia *et al.*, 2018) Potato can be directly consumed or processed in various forms such as fried and/or dried/frozen for later consumption. Nutritionally, it contains carbohydrates, proteins, vitamins B_1, B_3 and B_6, vitamin C and minerals like Potassium, Calcium and Iron. Potato is a major vegetable crop in the world, a source of starch and has a high calorific value. In addition, potato produces more food per unit area than any cereal crop within short periods and various consumer products are prepared from potato (FAO, 2013; Mishra *et al.*, 2020; Tadesse *et al.,* 2021).

2.2 Biology of potato plant

Potato *Solanum tuberosum* L. is an annual herbaceous plant reproducing both vegetatively by means of tubers and sometimes via botanical seeds. It belongs to the Kingdom Plantae, Division Tracheophyta, Class Magnoliopsida, Order Solanales, Family Solanaceae, Genus *Solanum* and Species *Solanum tuberosum* L. (Patil *et al.,* 2016). Recent estimates state that the Solanaceae family has more than 3000 species (Ganaie *et al.,* 2018). Besides potato, there are a number of botanical relatives which are alternate hosts to *P. infestans.* They include *capsicum* L. and eggplant *Solanum melongena* L., where tomato *Lycopersicon esculentum* (Mill.) is the major host crop (Lindqvist- Kreuze *et al.,* 2020).

2.3 Potato production in Kenya

Potato is mainly grown by small scale farmers as both food and cash crop in Kenya (Muthoni *et al.,* 2017). It is grown on 158,000 hectares per season, by about 800,000 farmers, with an annual production in two growing seasons of about 1.5 million tonnes (Muthoni *et al.,* 2017; FAO, 2017). In the developing countries, production has been increasing gradually since 1991, with equally increasing consumption (Walker *et al.*, 2011).

In Kenya, potato is majorly produced in high altitude areas of 1,200 to 3,000m above sea level, between 15 and 18℃ and soil pH of 5.5 to 6.0 (Janssens *et al.*, 2013; Muthoni and Kabira, 2015; Mutegi *et al.*, 2021). It is mainly grown the slopes of Mount Kenya, Aberdare ranges, Mau ranges and some other highland areas in Nyanza, Western and Trans–Nzoia regions. Small scale production is also done in Kericho, Kisii and isolated areas in Taita hills (Janssens *et al.*, 2013).

Table 2.1 shows the major counties ranked based on annual area under potato in Hectares (Ha) and annual production in Tonnes (Ton) in Kenya.

Table 2.1 Annual Potato Production by region

County	Acreage (Ha)	Production (Ton)
Kiambu	19,348	137,255
Nyandarua	45,740	409,105
Meru	17,414	130,102
Bomet	4,570	62,850
Nakuru	37,282	228,065
Elgeyo Marakwet	30,763	386,986
Nyeri	15,461	76,993
Taita Taveta	2,064	21,070
Narok	16,385	151,144
Uasin Gishu	1,545	16,943
Trans Nzoia	1,828	17,960
Laikipia	8,443	65,368
Murang'a	6,282	21,583
Baringo	3,522	62,795
Kajiado	1,452	10,266
West Pokot	960	10,560
Bungoma	1,776	38,495

(NPCK, 2017; MoALF&C 2020)

2.4 Challenges to potato production

In Kenya, potato production has not achieved its full potential despite its importance due to some biotic and abiotic constraints. These include; lack of clean certified seeds, poor soil fertility caused by poor management practices and intensive farming by farmers, pest and diseases (Shimira *et al*., 2020), seasonality in potato production, high cost of inputs such as fungicides and fertilizers, and marketing constrains (Riungu, 2011; Machangi *et al*., 2016). Many plant viruses (Abbas and Hameed, 2012; Abbas *et al.*, 2013; Gul *et al.*, 2013), nematodes

(Parveen *et al.*, 2013), bacteria (Ashraf *et al.*, 2012), and fungi (Guchi, 2015) have been documented as serious pests of potato.

Major fungal diseases of potato include late blight *Phytophthora infestans* (Mont.) de Bary, early blight *Alternaria solani* Sorauer, potato wart disease *Synchytrium endobioticum* (Schilb.) Percival, black scurf *Rhizoctonia solani* J.G. Kühn, silver scurf *Helminthosporium solani* Dur. and Mont., pink rot *Phytophthora erythroseptica* var. *erythroseptica* Pethybr., *Fusarium* dry rot *Fusarium* spp., and verticillium wilt *Verticillium dahliae* Kleb., and *Verticillium albo-atrum* Reinke and Berthold (Guchi, 2015).

According to Kroschel *et al.* (2020), there are nine major arthropod pest species affecting the potato crop in the Tropical and Subtropical regions. These are: Potato Tuber Moths (*Phthorimaea operculella* Zeller, *Symmetrischema tangolias* Gyen and *Tecia solanivora* Povolny (Lepidoptera: Gelechiidae)); Pea Leafminer Fly (*Liriomyza huidobrensis* Blanchard (Diptera: Agromyzidae)); Andean Potato Weevils (*Premnotrypes suturicallus* Kuschel, *P. vorax* Hustache and *P. latithorax* Pierce (Coleoptera: Curculionidae)); Potato Psyllid (*Bactericera (ex-Paratrioza) cockerelli* Sulc (Hemiptera: Triozidae)) and Bud Midge (*Prodiplosis longifila* Gagne (Diptera: Cecidomyiidae)).

2.5 Major fungal diseases of potato in kenya

Major fungal diseases of significant importance in Kenya are early blight *Alternaria solani* Sorauer and late blight *Phytophthora infestans* (Mont.) de Bary (NPCK, 2019). Early blight caused by *Altenaria* solani, is a common potato disease which may also occur on other *solanaceae* plants, such as pepper and eggplant, as well as certain Brassica. Although this disease usually affects older plants, it can cause complete defoliation under favorable environmental conditions. The disease mainly affects foliage and may occur on stem and tubers. The symptoms (Figure 4.3b) first occur on lower leaves as dark brown, oval or angular spots scattered on the leaf surface. A chlorotic zone usually surrounds the spots and may advance much beyond the lesion due to the presence of the toxin 'alternaric acid' produced by the pathogen. Under favorable environmental conditions, they enlarge rapidly to about 3-4 mm diameter, become irregular and may cover the entire leaf lamina (Binyam, 2014; APS, 2017; Robinson *et al.,* 2017).

De Vries *et al.* (2018), considers potato late blight as the most serious potato disease worldwide. It is also one of the major potato diseases in Kenya leading to up to 100% yield losses on susceptible varieties (Ongoro *et al.*, 2016).

2.5.1 Potato late blight *Phytophthora infestans*

Phytophthora infestans (Mont.) de Bary, the causal organism of late blight disease, belongs to the Kingdom Chromista, Division Oomycota, Class Oomycetes, Order Perenosporales, Family Perenosporaceae, and genus *Phytophthora*. *Phytophthora infestans* attacks members of the Solanaceae family, with potato *Solanum tuberosum* L., tomato *Lycopersicon esculentum* (Mill.) and eggplant (*Solanum melongena* L.) being its major hosts (Derevnina *et al.,* 2016; Petre *et al.,* 2021; Yuen, 2021).

Central Mexico is regarded as the pathogen's center of origin (Goss *et al.,* 2014), while other reports argue that the pathogen originated from Andes, South America (Gómez-Alpizar *et al.,* 2007). *Phytophthora infestans* is an oomycete, near bio-trophic, poly-cyclic, pathogen, which depends on cool humid environmental conditions for establishment. Its distinguishing characteristics are production of motile biflagellate zoospores and coenocytic mycelium (Arora *et al.,* 2014).

Phytophthora infestans mainly forms sporangia during the night, under humid conditions, which are dispersed during the day by wind under dry conditions (Arora *et al.,* 2014). It has branched sporangiophores produced by the mycelia form swellings from where they are produced and produce lemon-shaped sporangia at their tips. A sporangium releases three to eight zoospores at temperature of 12 to 15℃, whereas above 15℃ sporangia may germinate directly by producing a germ tube (Binyam, 2014; Shailbala and Kumar, 2017).

Phytophthora infestans requires two mating types, A1 and A2 in order to undergo sexual reproduction. Until the late 1980's both mating types had been reported only in Mexico. Lately, there has been a wide distribution of the two mating types in most countries resulting to development of new strains of the pathogen. The mating type A2 is absent in Kenya causing absence of sexual reproduction (Binyam, 2014; Shailbala and Kumar, 2017). De Vries *et al.* (2018), considers potato late blight as the most serious potato disease worldwide. It is also one of the major potato diseases in Kenya leading to up to 100% yield losses (Ongoro *et al.,* 2016).

2.5.2 Pathogen survival, spread and dissemination

Phytophthora infestans can survive in living host tissue, such as seed tubers, cull piles, volunteer potatoes that are left in the field off season, on alternate hosts crops and around the root zone in the soil (Kirk *et al*., 2013; Binyam, 2014; Johnson, 2020). Spores are readily spread among plants and fields by rain, overhead irrigation and wind (either directly or by the release of swimming zoospores) (Johnson, 2020; Yuen, 2021). The explosive disease potential of late blight is as a result of repeated cycles of spore production, dissemination in a single season (CropWatch, 2021).

Tuber infection can result to significant crop loss (CropWatch, 2021). Rains wash late blight spores down stem into the root rhizosphere, through the soil into the tubers (Johnson, 2020; Yuen, 2021). Spores washed from foliage and stems can infect tubers in the hill before harvest and spores present on foliage readily infect tubers during harvest (Figure 2.2) (Johnson, 2020; CropWatch, 2021). In the absence of a susceptible host, late blight results into a soil borne disease (Cooke *et al.,* 2011). Although tuber to tuber spread may occur in storage, its significance is not known (CropWatch, 2021). Figure 2.1 shows the infection cycle of late blight propagules on a potato field.

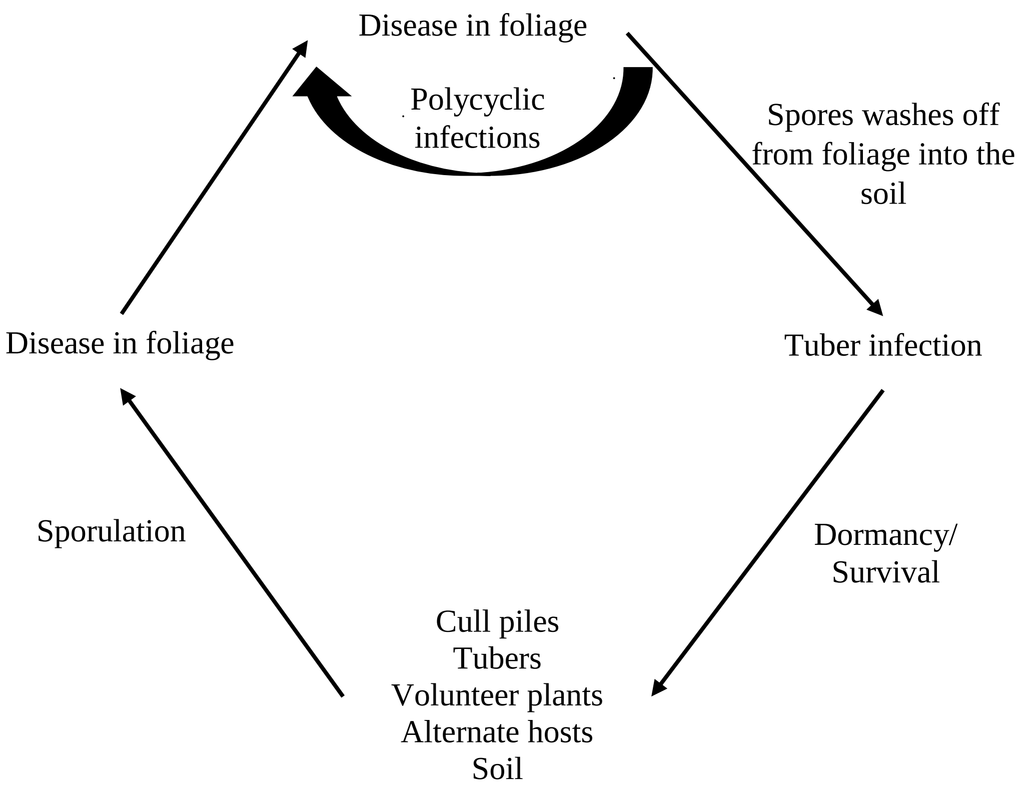


Figure 2.1 Infection cycle of late blight in potato

(CropWatch, 2021)

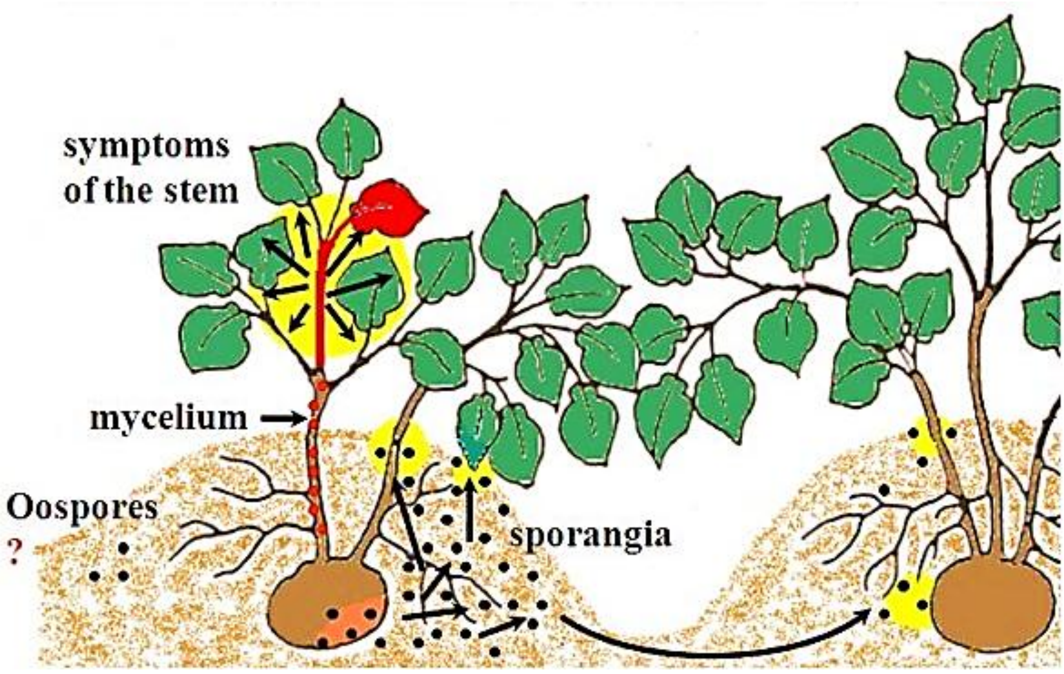


Figure 2.2 Development of late blight primary infections

(Zeller/Wagner JPS 3c)

2.5.3 Disease symptoms

Small, light-to-dark green, and circular to irregularly-shaped, water-soaked lesions are among the first symptoms of late blight in the field, which begin frequently at leaf tips and margins with a 'V' shape (Kirk *et al*., 2013; Robinson *et al.*, 2017), which usually appear first on the lower leaves where there is more humidity. However, they also occur on upper leaves under favorable conditions (Kirk *et al*., 2013; Binyam, 2014; Robinson *et al.*, 2017).

Lesions tend to enlarge rapidly in moist weather forming brown to blighted areas with indefinite borders. There is an appearance of a white zone of downy mildew growth of about 3-5 mm wide which appears at the border of the lesions on the abaxial side of leaves. This white growth forms a major distinguishing feature of late blight from other foliar diseases of potatoes. Soon the entire leaves are infected, die and become stiff (Robinson *et al.*, 2017; Greenlife, 2019).

Infection of the tubers by *P. infestans* may result from the tubers coming in contact with sporangia washed from stems and foliage lesions through the soil (Binyam, 2014). The infected tubers show superficial and irregular discoloration, and when cut open, they appear water-soaked and dark. Infected tubers may alternatively be covered with sporangiophores and spores of the pathogen, or invasion by secondary pathogens (Robinson *et al.*, 2017).

Figure 2.3 shows symptoms of potato late blight on foliage and tubers.

Figure 2.3 Symptoms of potato late blight

2.6 Management of fungal diseases in potatoes

To effectively manage fungal diseases in potato, various measures should be taken into consideration with the aim of reducing the primary inoculum and spread of the disease. These management practices are: cultural practices, chemical management, physical management and biological management (Rupp and Barry, 2017). Measures such as cultural practices significantly reduce the primary inoculum. Proper sanitary measures on planting materials and the field should be practiced to reduce the chance of introduction or re-introduction of late blight disease, avoiding injury the tubers during harvest, and proper fertilizer application. Other management measures are use of certified seeds Practicing crop rotation, inter-cropping with non-host crops, use of resistant varieties, timely planting and harvesting (Gevens and Seidl, 2013; Rupp and Barry, 2017).

If fungicides are applied in a timely manner, they can possibly slow down or even stop the development and spread of the pathogen (Binyam, 2014). Therefore, to be effective, there is need to apply fungicides before disease occurrence or on the appearance of the first symptoms (Binyam, 2014). Systemic or protectant fungicides such as copper salts, formaldehyde, dithiocarbamate, and metalaxyl can be used for effective management of fungal pathogens (Anwar *et al.*, 2015).

The use of biological agents (BCAs) (rhizosphere resident microbial antagonist) (esp. *Trichoderma* sp., *Pseudomonas* sp. and *Bacillus* sp.) (Lal *et al.,* 2016; Tadesse *et al.,* 2021) and botanicals (esp. *Melia azedarach, Lantana Camara, Azadirachta indica, Pistacia lentiscus and Datura* Sp. among others) (Lal *et al.,* 2016; Messgo-Moumene *et al.,* 2017; Nagar *et al.,* 2017; Yadav *et al.,* 2017) as management strategy has proven to be promising in plant disease management. In the recent years, there is more ongoing research along the field of biological control (Lemessa, 2006; Henok *et al.,* 2007; de Vries *et al.,* 2018).

Biological agents and botanicals possess competitive, inhibitory and suppressive effects (Cizcova *et al.,* 2002) creating a hostile environment to the development fungal pathogens in potato. The antimicrobial compounds constituted in some plants have various mechanisms of action against plant pathogens, which include induced host resistance and growth inhibition with substances such as phenols, proteins, saponins, glycoprotein, flavanones and alkaloids among others. Fungal antagonists also employ various mechanisms to protect plants by production of antibiotics, competition for food and space, and inducing plant resistance (Sanda and Sunusi, 2014; Lal *et al.,* 2016).

An interesting concept to explore is the integrated disease management (IDM). It involves the use of a combination of either two or more of the management strategies such as resistant potato varieties, chemical fungicides and cultural control measures. This combination offers the best option for management of disease in the tropical highlands of Africa (Guchi, 2015). To prevent disease spread via seed, all stored potatoes should be scouted frequently and diseased tubers removed from storage (Stone, 2019).

2.6.1 Use of plant extracts in management of fungal diseases on potato

Most bioactive compounds are primarily derived from plants (Altemimi *et al*., 2017; Loi *et al.,* 2020), which are further classified into primary or/and secondary metabolites (Sharma *et al.,* 2019). There is increasing research on the antifungal properties of plant extracts around the world (Khan and Nasreen, 2010). Research on plant extracts has been intensified in the recent years because they are eco-friendly and cost effective (Nagar *et al.,* 2017), and have been found to contain secondary metabolites that are effective in disease control (Gurjar *et al.,* 2012). According to Enyiukwu *et al.* (2014) and Nagar *et al.* (2017), plant extracts have exhibited a wide range of activity against plant pathogens. Altemimi *et al*. (2017) considered plant extract as good source of natural antioxidants and antimicrobials after they realized strong antioxidant potential both *in vitro* and *in vivo* exhibited by the extracts. Tables 2.2 and 2.3 below shows the phytochemicals extracted by ethanol and acetone and the mechanisms of action exhibited (Gurjar *et al.*, 2012).

Extracts from several plant species have been tested for inhibitory effects against *Phytophthora infestans*. Some include weeping willow *Salix* sp. leaf extracts and thorn apple *Datura* sp. causing maximum growth inhibition of 59.80 and 48.37% on *P. infestans*, respectively (Nagar *et al.*, 2017). Yadav *et al.* (2017) reported the Neem extracts from *Azadirachta indica* Juss., reduced late blight severity by 43.31%. Messgo-Moumene *et al.* (2017) also realized a significant growth inhibition by extracts from pomegranate *Punica granatum* L., bark at 90% and mastic tree *Pistacia lentiscus* L. leaves and berries at 88%.

There are a number of factors limiting the activity of extracts from botanically active plants. These include; environmental factors, choice of solvent, source of the organisms, biochemistry, physiology, metabolism and adaptation strategies of the microbes, plant species, biochemistry, age and parts, concentration of the plant extract and period of extraction. (Izah, 2018; Yuan *et al*., 2020)

There are exists various techniques for crude extracts extraction from plant material, the most commonly used include; Soxhlet extraction, maceration, and hydro-distillation (Loi *et al.*, 2020). An array of solvents is also used to extract various compounds; however, the choice of solvent is dependent on the type of plant, part of the plant to be used for extraction, nature of the bioactive compounds, and the availability of solvent (Altemimi *et al*., 2017). Generally, polar solvents such as water, methanol, and ethanol are used in extraction of polar compound, while nonpolar solvents such as hexane and dichloromethane are used in extraction of nonpolar compounds (Abubakar and Haque, 2020).

Table 2.2 Solvents used for phytochemical extraction

Phytochemicals	Ethanol	Acetone
Flavonols	+	+
Phenols	+	+
Saponins	-	+
Alkaloids	+	-
Tannins	+	-
Terpinoids	+	-
Polyacetylenes	+	-
Sterols	+	-

Source: (Gurjar *et al.*, 2012; Pandey and Tripathi, 2014)

Table 2.3 Phytochemicals and their modes of action against plant pathogens

Class	Sub-class	Mechanism
Phenolics	Simple phenols	Membrane disruption, substrate deprivation
Phenolic acids	Phenolic acids	Bind to adhesins, complex with cell wall, inactivate enzymes
Terpenoids, essential oils	-	Membrane disruption
Alkaloids	-	Intercalate into cell wall
Tannins	-	Bind to proteins, enzyme inhibition, substrate deprivation
Flavonoids	-	Bind to adhesins, complex with cell wall, Inactivate enzymes

(Source: Gurjar *et al.*, 2012)

2.6.2 Use of antagonistic fungi in management of foliar diseases on potatoes

Microbial biocontrol agents (BCAs) for plant diseases are usually fungal or bacterial strains isolated from the phyllosphere, endosphere or rhizosphere with great potential in managing plant-pathogenic organisms (Thambugala *et al*., 2020).

Fungal antagonists have, from several published research, employed various mechanisms of action against plant-pathogenic organisms (Figure 2.4). some of the key mechanisms include; *antibiosis* -production of an inhibitory metabolite or antibiotic-, *mycoparasitism* -deriving some or all of nutrients from the fungal host-, *induced resistance* -induction of plant defense response against plant pathogens- and *growth enhancement* -BCAs promote plant growth while the effects of the disease are being reduced and also through microbial hormones such as indoleacetic acid and gibberellic acid-. Other actions involved include; secretion of extracellular hydrolytic enzymes by the antagonist, competition for space and nutrients between organisms and detoxification of virulence factors (Zhang *et al*., 2014; Deketelaere *et al*., 2017; Köhl *et al.*, 2019; Thambugala *et al*., 2020).

Recently, there has been a great increase in the potential for application of fungal biological control agents against plant pathogens. This is because fungi have a comparatively high reproductive rate both sexually and asexually, a short generation time and they are target specific. Additionally, in the absence of the host, fungi can survive in the environment shifting their mode of parasitism to saprotrophism therefore preserving sustainability. Several fungal species have mechanisms (Figure 2.4) which allow them to efficiently protect plants from diseases resulting from plant pathogenic fungi (Thambugala *et al*., 2020).

Over time, several fungal antagonists have been used to manage foliar diseases on potato. An experiment was carried out by Soytong and Ratanacherdchai (2005) using *Chaetomium mycofungicide,* a formulation from *Chaetomium cupreum* strains CC01-CC10 and *Chaetomium globosum* strains Cg1-Cg12t. They reported a significant reduction of potato late blight symptoms upon regular spraying at a concentration of 1.5 x 10^6 CFU/g, and an application rate of 0.5 g/L. They further recommended that crude extracts of *Chaetomium* sp. and *Trichoderma* sp., could induce plant immunity.

El-Naggar *et al.* (2016) investigated the efficacy of *Trichoderma harzianum* Rifai and *Trichoderma viride*,Pers., in controlling *P. infestans*. *Trichoderma harzianum* highly suppressed the growth of *P. infestans* by 83.3%, whereas *T. viride* suppressed the growth of *P. infestans* by 75.1%. Their findings were not different from those by other researchers.

Chowdappa *et al.* (2013) also reported that *T. harzianum* OTPB3 showed mycelial inhibition of *P. infestans*. According to Zaker *et al*. (2016), *T. viride* inhibited the radial growth of *P. infestans in vitro* by 36.7% and completely overgrew *P. infestans* colony, whereas *P. fluorescens* inhibited *P. infestans* by up to 88%.

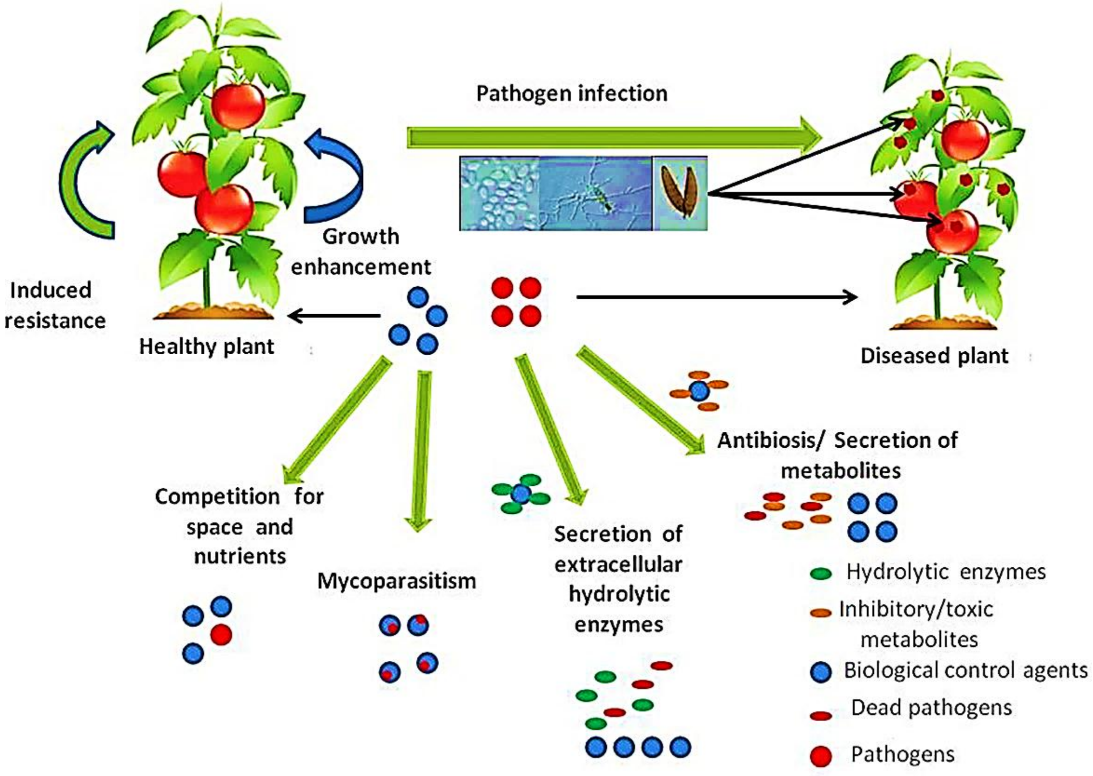


Figure 2.4 Key mechanisms of action involved in biological control of plant fungal diseases by fungal antagonists.

(Thambugala *et al*., 2020)

Despite the existing and well proven advantages of fungal antagonists in management of plant disease, there exists a number of limitations which include; environmental factors i.e., extreme temperatures, UV and desiccation, competition for food and space from other microorganisms, slower effect compared to synthetic fungicides ((esp. for hyperparasites) due to time needed for establishment and colonization to supress and/or kill the pathogen), incompatibility with other management options such as chemical control (Pertot *et al*., 2016; 2017)

2.7 Mass multiplication of antagonistic fungi

In order to develop new biological products to manage plant pathogens, there is need for large scale screening of candidate antagonists, developing mass production protocols that optimize product quantity and quality, and devising a product formulation that preserves, helps product delivery and enhances bioactivity (Mulatu *et al.*, 2021). According to Janisiewicz *et al.* (2013), lack of appropriate methods for mass production of bio-agents is one of the greatest obstacles to application of biological control in crop disease management.

Mass production fungal propagule is achieved through solid and liquid fermentation techniques. The techniques are Solid-state fermentation (SSF) and liquid state fermentation or submerged fermentation (SmF), where the SSF is preferable (Naeimi *et al.*, 2020). Solid state fermentation involves the cultivation of microorganisms on an organic solid substrate, which is generally a non-soluble material that serves as physical support as well as nutrient source, while Liquid state fermentation (LSF) is applied for the processes in which soluble materials in water is used for the microbial growth (Babu and Palavi, 2013; Naeimi *et al.*, 2020).

Trichoderma spp. have been multiplied on solid substrates such as sorghum grain, wheat straw, wheat bran, spent tea leaf waste, coffee husk and saw dust. Zaid and Singh (2004) and Janisiewicz *et al.* (2013) mass multiplied *Trichoderma harzianum* on pre-soaked and autoclaved seeds of *Echinochloa frumentacea* Link (Indian barnyard millet, sawa millet or billion-dollar grass) for 12 days at 28℃. The sawa millet seeds were air dried, and ground and simultaneously passed through 50 and 80mm mesh sieves to obtain the spore powder. For commercial formulations, ground powder was diluted with talcum powder containing 1% carboxymethyl-cellulose to obtain a desirable concentration of the biocontrol agent.

The first major concern in large scale production systems is the attainment of suitable growth of the biocontrol agent. Biomass production is a major challenge due to the specific requirement of nutritional and environmental conditions for the growth of organism (Kumar *et al.*, 2014).

There is therefore high potential for use of biocontrol products such as fungal antagonists and plant extracts for management of potato late blight *P. infestans*. There is also need for prospecting, identification and formulation of more bioactive plants extracts and microbial isolates for the development of bio-pesticides for late blight disease. This study aimed to develop effective microbial and botanical based bio-pesticides for managing late blight on potato.

CHAPTER THREE: MATERIALS AND METHODS

3.1 Assessment of the effects of the socio-demographic factors, production and management practices of potato and prevalence of late blight on potato in Nyandarua County

3.1.1 Survey study site description

A baseline survey was carried out in Kinangop Sub-County, Nyandarua County (Figure 3.1) in May 2018 in order to assess the influence of production practices on the occurrence of potato late blight. The area is a major potato growing region, where potato is ranked the most important commercial crop (Kaguongo *et al*., 2010).

Nyandarua County is located west of the Aberdare ranges lying in the Upper Highland sub-zone 3 (UH3) Agro-ecological zone (Jaetzold *et al.,* 2007), at latitude 0°23'57.88"S and longitude 36°29'22.26"E. The County experiences bimodal rainfall: with long rains from March to May with a maximum rainfall of 1,600 mm and short rains from August to December with a maximum rainfall of 700 mm. The average annual rainfall is 1,500mm. the soil classes found in Nyandarua County are; clay, clay loam, loam, sandy clay, sandy clay loam, sandy loam, silt loam and silty clay loam (CGSpace, 2021).

Kinangop receives higher amounts of rainfall while Ndaragwa and Ol'Kalou receive comparatively lower rainfall. Frost occurs for the last few hours before sunrise at temperatures ranging between 1 and 2 degrees Celsius (℃) and its effects are felt more in zones bordering the Aberdare Ranges. December records the highest temperature with a mean average of 21.5℃ while July has the lowest with a mean average of 7.1℃ (CIDP, 2018).

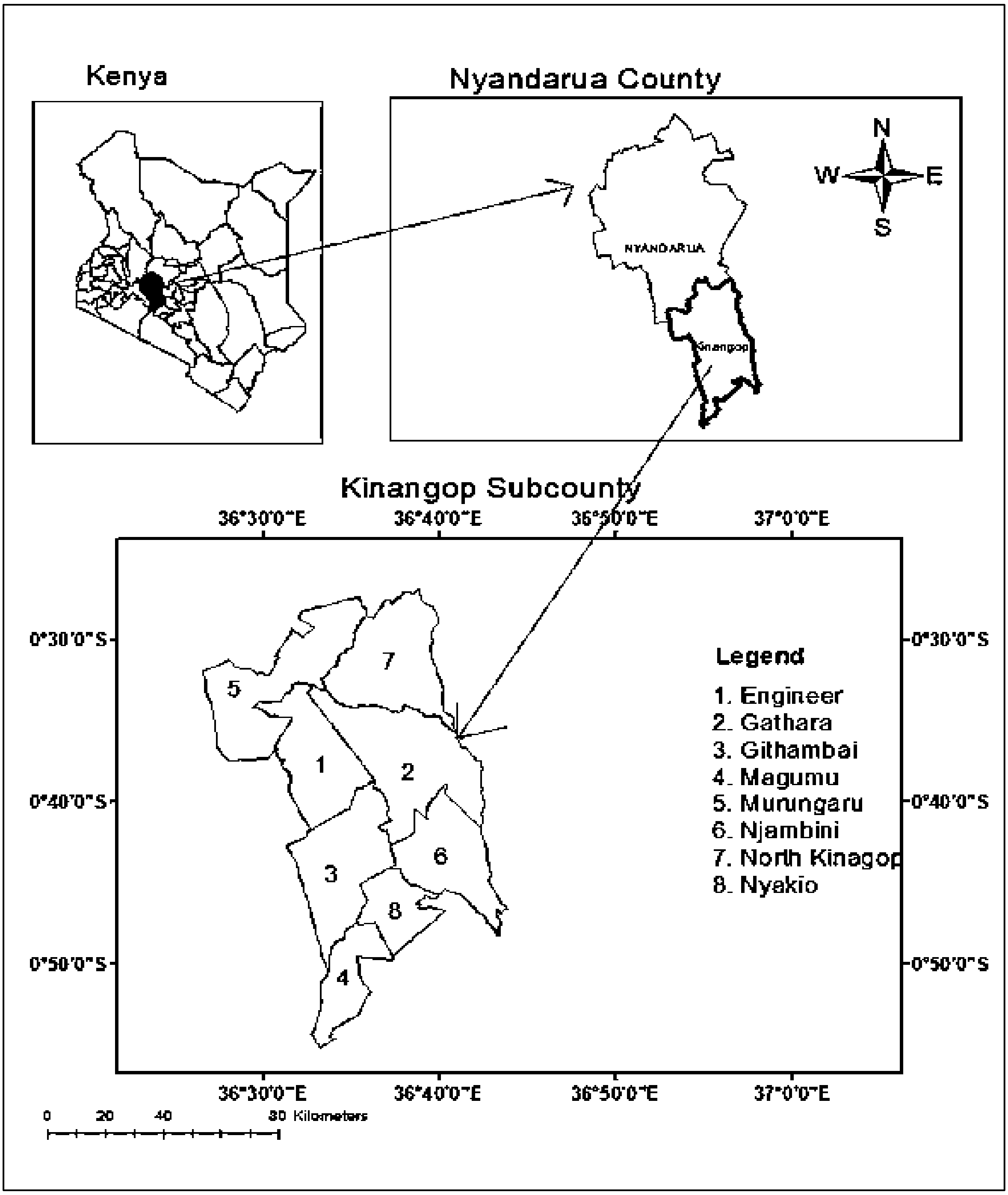


Figure 3.1 A map of Nyandarua County, Kinangop Sub-County showing the wards where the survey study was carried out

(Source: Google maps)

3.1.2 Sample size and sampling design

For sampling, the guideline by Mugenda and Mugenda (2003) was used. Accordingly, the largest margin of error, 8%, gave a total of 156 respondents from 143,879 households at 0.05% significance level. Total number of households was used to derive the sample size since there is no existing information on the exact number of small-scale potato farmers in Nyandarua County. This sample size was arrived at by Solvin's formula as used by Subianto and Hamsal, (2013).

$$\boldsymbol{n = \frac{N}{1 + Ne^2}}$$

Where: $\boldsymbol{n}$ is the required sample size; $\boldsymbol{e}$ is the margin of error (8% at 95% confidence level) and $\boldsymbol{N}$ is the total population.

Purposive random sampling method was used to sample only small-scale potato farmers with less than 3 ha under potatoes and at least 1km apart. The survey was conducted by administering open-ended questionnaires (Appendix I) to the achievable 105 households' heads (the achievable number) or representatives in the eight wards of Kinangop Sub-County. The questionnaire contained open-ended questions allowing the respondents to fully explain themselves, so as to gather more elaborate information (Farrell, 2016).

The distribution of the respondents was as follows, Engineer 20, Njabini 28, Gathara 17, Gathabai 7, North Kinangop 2, Magumu 19, Murungaru 3 and Nyakio 9. The surveyed farms were spread between 2,415 and 2,684m above sea level (asl).

The interviews were conducted at the respondents' farms to capture and confirm the information on the size of the farm, the area allocated to potato farming, farming history, cropping systems practiced, and late blight disease occurrence and management strategies. Photo cards were used to enable respondents to easily identify the symptoms of various diseases/pests observed in the farms (Schneider *et al.*, 2013).

3.2 Determination of antifungal activity of antagonistic fungi against *P. infestans* under laboratory conditions

3.2.1 Collection of soil samples and diseased plant materials

Representative soil samples were collected from random potato farms in Nyandarua and Kiambu Counties. A zig-zag pattern was used to locate the sampling points in each farm after which the soil was drilled and scooped using soil auger to a depth of 15cm around the potato rhizosphere. Three representative soil samples were picked from each surveyed farm and properly mixed in a bucket where about 50g of the composite sample was packed in khaki bags, labeled appropriately (place, date of collection and crop at the surveyed fields) then transported in a cool box to Kenyatta University Agricultural laboratory for storage. Once in the laboratory, the soil samples were air dried on benches for seven days for further screening.

Tissue samples with typical symptoms of *P. infestans* were randomly collected from various infected potato plants, kept in plastic containers, labeled appropriately (place, date of collection and the name of host variety at the surveyed fields) and then transported in a cool box to Kenyatta University Agricultural laboratory for storage and subsequent assessment.

3.2.2 Isolation and identification of *Phytophthora infestans*

Infected potato leaves were gently washed under running tap water, dried and cut into small pieces at the periphery of the infected area. The leaf pieces were incubated under a sterile potato tuber slice of about 5mm thick in a sterile 9cm diameter disposable petri dish with moistened filter paper at 24±2℃ until fresh sporulation appeared as described by Siameto *et al.* (2010). Alternatively, to obtain more spores, infected leaf samples were washed under tap water, dried and kept in a humid chamber for fresh sporulation. The freshly sporulated mycelia were carefully picked without touching the tuber or leaf surfaces and inoculated on carrot sucrose agar media (fresh carrot infusion 220g/L; Sucrose 20g/L; Agar 9g/L) then incubated at 24±2℃ for seven days. *Phytophthora infestans* was identified based on morphological characteristics after examination under a compound microscope (XSZ-107T) at a magnification of ×40, and identified as described by Erwin and Ribeiro (1996).

3.2.3 Isolation and purification of fungi from soil and plant samples

Isolation of microbial antagonists was carried out using serial dilution as described by Fulano *et al.* (2016). One gram of each composite soil sample was added to a universal bottle containing 10 milliliters sterile distilled water and shaken to get the first stock solution. One milliliter from first stock solution was transferred into 9 milliliters sterile distilled water in a universal tube to get a second stock solution of dilution factor, this was done to a third dilution factor. One milliliter of the final dilution was transferred into media plates in triplicate. The plates were swirled gently to mix the suspension uniformly, sealed with a cling film and incubated (BJPX-H230JI) at 24±2℃ for seven days.

The isolation of microorganisms from plant materials was achieved by direct plating method. The plant parts were washed in running tap water and then surface sterilized with 1% sodium hypochlorite for three minutes and then cut into small pieces and directly plated on potato dextrose Agar (PDA) in a 9 cm diameter disposable petri dish. The cultures were thereafter incubated for seven days at 24±2℃. After incubation (BJPX-H230JI), a mixed culture was attained (Plate 3.1-A) and those fungi seen to suppress the mycelial growth of other fungi were sub-cultured and purified on PDA (Plate 3.1-B; C; D) under a laminar flow hood (BBS-H1100). Purified fungal cultures were maintained on PDA slants (Figure 3.2) at 4℃ until further use (Killani *et al.*, 2011).

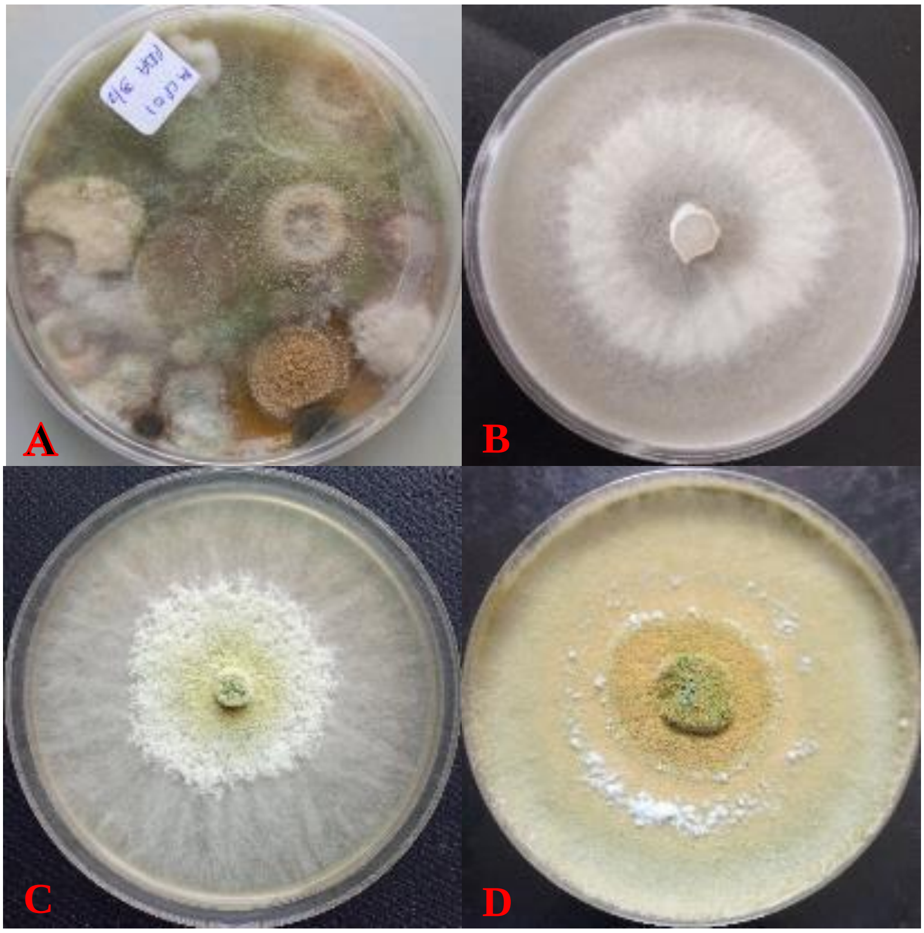


Plate 3.1 Some of the fungal cultures isolated from potato rhizosphere

***A** = Mixed culture, **B, C** and **D** = Pure cultures of *Trichoderma* spp. isolates

Figure 3.2 Agar slants prepared for preservation of pure fungal cultures

3.2.4 Identification of isolated fungi

For identification of the isolated fungi, each isolate was aseptically cultured on PDA (potato infusion 200g/L; Dextrose 20g/L; Agar 15g/L). amended with 100mg/L of tetracycline (an antibiotic to inhibit bacterial growth). This was aseptically conducted under a laminar flow hood (BBS-H1100). Fungi was identified based on their colony appearance. A slide culture was prepared by carefully picking a segment of sporulated mycelium from a petri dish and placing on glass slides containing a drop of molten PDA medium (45℃) then covering with cover slides and incubating for growth. Microscopic identification was done by staining a 7 days old slide culture using lactophenol-cotton blue (LCB) and examined under a compound microscope (XSZ-107T) at a magnification of ×40 based on the shape of conidia and conidiophores and branching pattern of phialides (Nelson *et al.*, 1983).

3.2.5 *In vitro* antagonistic activity of isolated fungi against *P. infestans*

The assay for antagonism was carried out on Pea agar medium (fresh pea infusion 120g/L; Agar 15g/L; sucrose 7g/L) using the dual culture technique (Plate 3.2) under a laminar flow hood (BBS-H1100). A mycelial plug of *P. infestans* was cut with 5 mm sterile cork borer to obtain agar discs which were placed at the center of a 9cm Petri dish containing PDA, and pre incubated at 24±2℃ for 2 days to initiate fungal growth. Mycelial plugs were aseptically cut from the fungal antagonist and inoculated on the pre-incubated Petri dish at four equidistant positions 3 cm from the *P. infestans* mycelial plug.

A *P. infestans* mycelial plug to be used as control was placed at the center of petri dish (9cm diameter) containing PDA and incubated at 24±2℃. All the pairings were carried out in triplicates and incubated at 24±2℃ for ten days to allow adequate interaction between the antagonists and the pathogen. The plates were arranged in a completely randomized design (CRD) on sterile benches (Fulano *et al.*, 2016).

To monitor the gradual growth over time, antagonistic activity was recorded on the 4th, 6th, 8th and 10th day after incubation by measuring the colony diameter (cm) of the *P. infestans* mycelial growth in the control plate (D_c) and the colony diameter of the *P. infestans* mycelial growth in the Plate with the antagonists (D_t). The colony diameters were determined by the mean of two perpendicular diameters. The two readings were transformed into percentage growth inhibition (PGI) by modification of the formula used by Fulano *et al.* (2016):

$$PGI = \frac{D_c - D_t}{D_c} \times 100$$

Where: ***PGI*** is the percentage growth inhibition, D_c is the diameter of the *P. infestans* colony in the control Plate, and D_t is the diameter of the *P. infestans* colony in the plate with the antagonist (Killani *et al*., 2011).

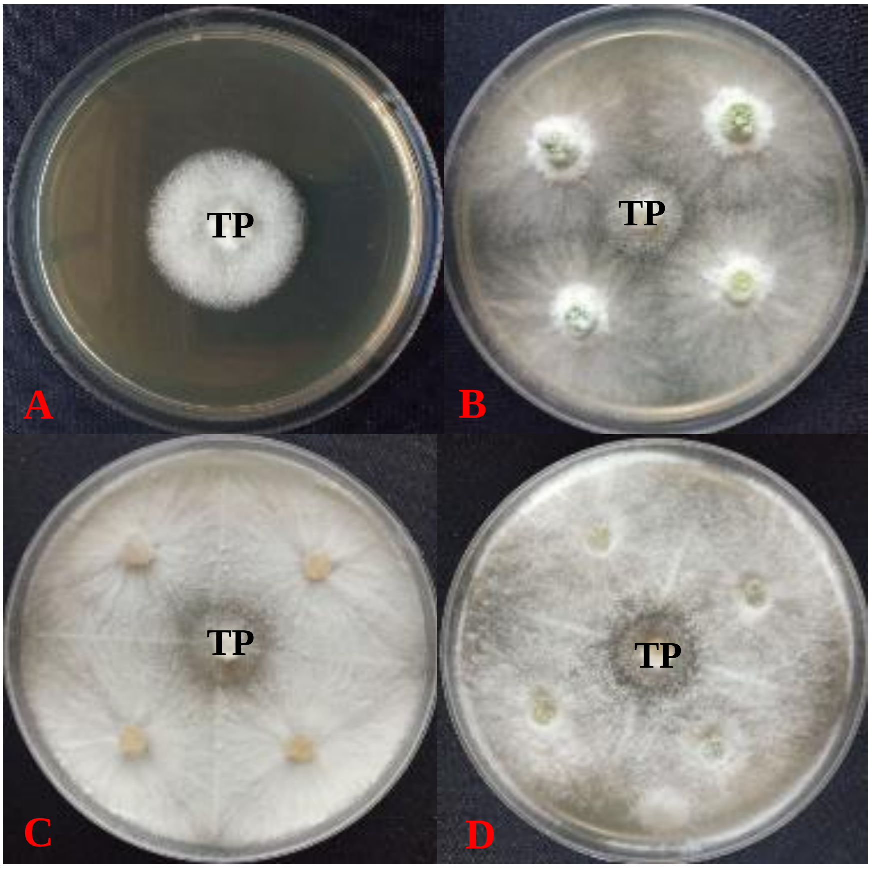


Plate 3.2 Antagonistic activities of some active fungal isolates against *P. infestans* using dual culture technique

***A** = Control; **B** = *T. hamatum*; **C** = *T. asperellum*; **D** = *T. afroharzianum*; **TP** = Test pathogen, *P. infestans*; (7 days old cultures).

3.3 Identification of plants with bioactive potential, extraction and evaluation against *P. infestans in vitro*

3.3.1 Collection and preparation of bioactive plant materials

Locally available plants with medicinal potential were identified based on ethnobotanical assessment (Ahmed *et al.,* 2020) (Figure 3.3, 3.4 and 3.5). Eleven plants were then collected from the Kenyatta University (KU) botanical garden for the *in vitro* evaluation of the suppressive potential against *P. infestans* (Goufo *et al.*, 2010; Al-Samarrai *et al*., 2012). The following plants Table 3.1) were collected: Neem *Azadirachta indica* (A.) Juss, Mastic tree *Pistacia lentiscus* L., Mexican sunflower *Tithonia diversifolia* (Hemsl. A.) Gray, Mexican marigold *Tagetes minuta* L., European cocklebur *Xanthium strumarium* L., Sodom apple *Solanum incanum* L. Jimson weed *Datura stramonium* L., Lantana *Lantana camara* L., Lemon grass *Cymbogopon citratus* (Dc.) Stapf, Devils horsewhip *Achyranthes aspera* L. and Lemon bottlebrush *Callistemon citrinus* (Curtis).

Appropriate plant samples (leaves and fruits) were collected and air dried at 24±2°C for 7 to 21 days. After drying, the samples were chopped into small pieces and ground using an electric grinder. The ground fine powder was stored in khaki bags at 24±2°C until further use (Mohana and Reveesha, 2007; Din *et al.,* 2016).

Table 3.1 Photos of plant materials collected from the Kenyatta University botanical garden

	
Mexican sunflower *Tithonia diversifolia*	Mastic tree *Pistacia lentiscus*
	
Neem *Azadirachta indica*	Lemon bottlebrush *Callistemon citrinus*

	
Mexican marigold *Tagetes Minuta*	Sodom apple *Solanum incanum*
	
European cocklebur *Xanthium strumarium*	Jimson weed *Datura stramonium*
	
Lantana *Lantana camara*	Lemon grass *Cymbogopon citratus*
	
Devils horsewhip *Achyranthes aspera*	

3.3.2 Extraction of crude extracts from the plant materials

Extraction of the crude extracts from the collected plant materials was done using maceration technique as described by Mohana and Reveesha (2007) and Farooq and Nasreen (2015) with slight modifications. Finely ground plant materials were mixed with two solvents ethanol and acetone (1:1 vol/vol) at a ratio of 1:10

(wt/vol, dry powder/solvent), in a conical flask. The mouths of the flasks were tightly plugged with cotton wool and covered with aluminum foil.

Conical flasks containing the various plant samples were stored for 24hrs with periodic agitation. The solution was subsequently filtered through four-layered serviettes and filtered through Whatman filter paper No. 1 to give an extract of crude concentration. A rotary evaporator was used to extract the two solvents at 60℃ (Boiling point of Acetone: 55℃; Ethanol: 78℃) from the crude extract. The resultant aqueous plant extracts were aseptically stored at 4℃ in glass bottles (Swapna and Lalchand, 2013). Table 2.2 shows ethanol and acetone, solvents used in extraction and the types of phytochemicals reportedly extracted by the solvents from previous studies.

3.3.3 Evaluation of the bioactive compounds against *P. infestans in vitro*

Poisoned food technique (Plate 3.3; B, C and D) was used for the *in vitro* evaluation of the plant extracts against *P. infestans* as described by Farooq and Nasreen (2015) and Messgo-Moumene *et al.* (2017). Two milliliters of each crude extracts were poured into conical flasks containing molten 200 milliliters carrot sucrose agar (45℃). The content in the conical flask was gently swirled to attain a uniform mixture then dispensed on sterile petri dishes (9cm diameter). Media un-amended with plant extracts was dispensed in petri dishes and considered as control. A 5mm mycelial plug from *P. infestans* culture was placed at the center of every petri dish containing media amended with plant extracts and, in the control, plates then incubated at 24±2℃ in a CRD design.

Growth suppression (GS) was recorded on the 4th, 6th, 8th and 10th day after incubation by measuring the colony diameter of the *P. infestans* mycelial growth in the control (D_c) and in the plates amended with the plant extracts (D_t). The colony diameter was given by the mean of two perpendicular diameters. The two readings were transformed into percentage growth suppression (PGS) using the formula used by Fulano *et al.* (2016) with slight modifications:

$$PGS = \frac{D_c - D_t}{D_c} \times 100$$

Where: ***PGS*** is the percentage growth inhibition, $\boldsymbol{D_c}$ is the diameter of the *P. infestans* colony in the control, and $\boldsymbol{D_t}$ is the diameter of the *P. infestans* colony in the plate with the plant extracts (Killani *et al.*, 2011).

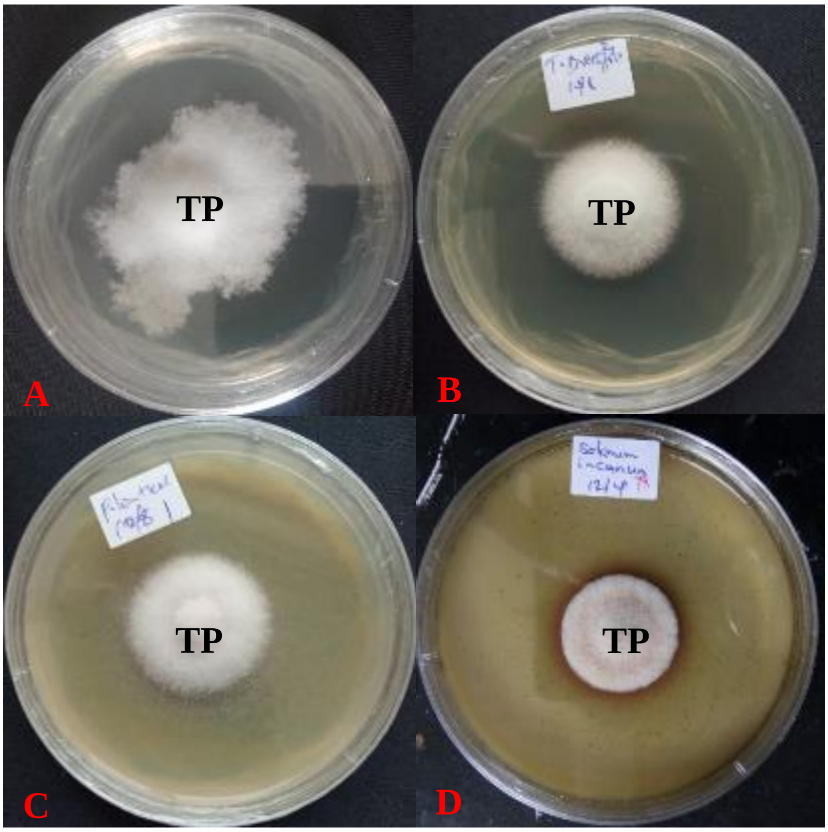


Plate 3.3 Antagonistic activity of some bioactive plant extracts against *P. infestans* using poisoned food technique

***A** = Control on PDA; **B** = *T. diversifolia*; **C** = *P. lentiscus*; **D** = *S. incanum*; **TP** = Test pathogen (*Phytophthora infestans)* (7 days old cultures)

3.4 Evaluation of fungal antagonists and plant extracts in managing potato late blight in the field

3.4.1 Field evaluation study site description

The experiment was carried out at Kenya Agriculture and Livestock Research Organization (KALRO)–Tigoni, about 4km from Limuru town, Kiambu County, Kenya (Figure 3.6) in three seasons. The first season was carried out from May 2018 to October 2018, the second season from December 2018 to March 2019 and season three from April 2019 to July 2019. The geographical coordinates of the study site are 1°08’56.56” S and 36°41’6.86” E with an elevation of about 2100m above sea level. KALRO-Tigoni experiences an average temperature of 26℃ and average annual bimodal rainfall of 1,200 mm. The research center has sandy clay soils, with pH ranging between 4.6 and 4.8. The experiment was conducted on fields naturally infested with *P. infestans* inocula based of the farm history.

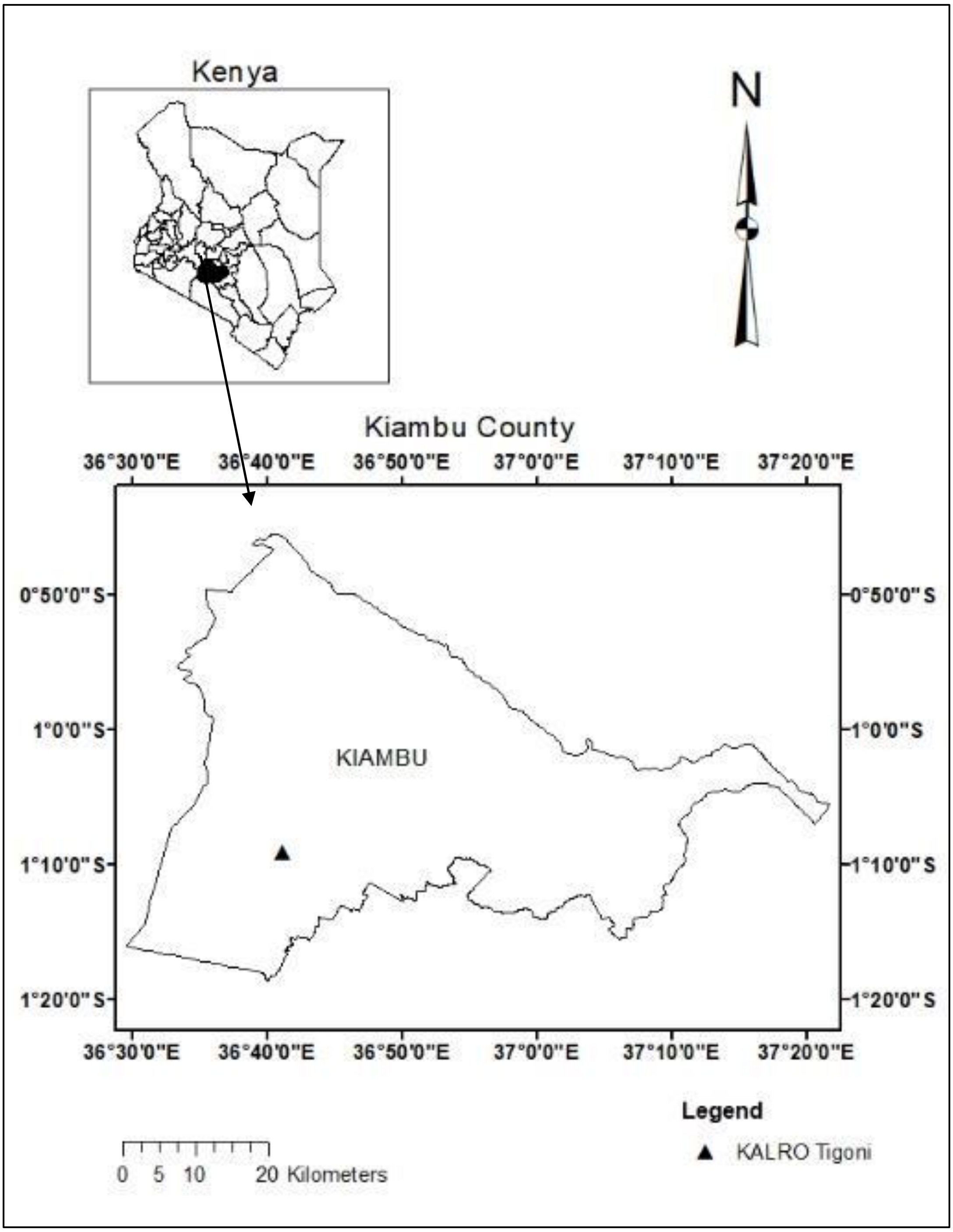


Figure 3.3 A map of Kiambu County showing the location of KALRO Tigoni Horticulture Research Center where field evaluations were conducted.

(Source: Google maps)

3.4.2 Soil physicochemical analysis

Soil samples were collected from the field evaluation site (KALRO-Tigoni) prior to experimental set up. Top soil (15cm) was collected in a zig zag pattern and mixed to obtain composite and composite sample. The soil samples were carefully packaged and taken for analysis of physical and chemical properties at the National Agricultural Research Laboratories (NARL), Kabete. Soil texture, percentage sand, silt and clay, was analyzed using the Bouyoucos Hydrometer method and classified using a textural classification chart of Klute (1986). The bulk density, particle density and percentage porosity of the soils were also analyzed based on the methods used by Hinga *et al.* (1980).

Mehlich Double Acid method was used to analyze the available nutrient elements (Phosphorus, Potassium, Calcium, Sodium, Magnesium and Manganese) in the soils (Mehlich *et al.,* 1962). Kjeldahl method was used to determine the total Nitrogen (Page *et al.,* 1982). Total organic carbon was analyzed using Calorimetric method (Anderson and Ingram, 1993). Trace elements Iron, Zinc and Copper were extracted with 0.1 M HCl (Mehlich *et al.,* 1962). Soil pH (Soil pH – water) and Exchangeable Acidity (for soils with pH < 5.5) was measured as described by Mehlich *et al.* (1992), Anderson and Ingram (1993) and Okalebo *et al.* (2002).

3.4.3 Experimental layout and design

On farm experiments were set up KALRO–Tigoni in Limuru, Kiambu County in two long rain seasons and one short rain season. The field was laid out in a Randomized Complete Block Design (RCBD), with 4 replicates per treatment. Treatments included *Trichoderma harzianum, Trichoderma afroharzianum, Trichoderma asperellum, Azadirachta indica, Pistacia lentiscus, Tithonia* *diversifolia,* Mistress® (synthetic fungicide) and water. Two potato varieties Shangi and Tigoni were planted. According to NPCK (2017; 2019) Shangi is a moderately susceptible variety while Tigoni is tolerant to late blight. Clean certified potato seed tubers were sourced from KALRO-Tigoni research station and planted on plots measuring 4 × 3m at the recommended spacing of 30 × 75cm, 60cm alley between the plots and 60cm alley between the blocks (Muthoni and Kabira, 2011). Standard agronomic practices (proper soil preparation, appropriate fertilization, pest and disease management etc.) were carried out till harvesting (Hassen *et al.,* 2015).

3.4.4 Mass multiplication and formulation of biocontrol agents (BCAs)

The fungal antagonists showing greatest antagonistic activity on *P. infestans in vitro* were mass multiplied using sorghum grains as a substrate as described by Fulano *et al.* (2016). Four hundred milliliters of distilled water were added in a 1L conical flask containing 200g of sterilized sorghum seeds pre-soaked with 2% sucrose and further soaked overnight. After soaking, the excess water was drained out and the sorghum reconstituted with 50 millilitres of clean water then autoclaved (UTKBS-V series) at 121℃ for 15 minutes. After autoclaving the sterile sorghum was allowed to cool and transferred into sterile polythene sleeves.

Seven days old fungal cultures of the antagonists were gently scrapped and put in the sorghum substrate in the polypropylene sleeves in a lamina flow hood (BBS-H1100) as described by Martinelli *et al.* (2012). Periodic agitation was ensured in order to evenly distribute the inocula, prevent aggregation and to ensure proper aeration for sufficient sporulation (Sivakalai and Ramanathan, 2015). The substrate was incubated for 14 days at 24±2℃.

Sorghum grains colonized by fungal antagonists was aseptically dried and ground to powder. To determine the spore density, 1g of the inoculated dry sorghum was mixed with 10 millilitres of sterile water then conidial suspension obtained by filtering the suspension through a two layered cheese cloth. A drop of the filtrate was placed on a Neubauer haemocytometer slide under a compound microscope (XSZ-107T) at ×40 magnification, spores counted and recorded followed by standardisation to 1.0×10^8 spores/ml to get the desired density of the BCAs. The dry ground powder was mixed with Talc powder (1:2w/w) and Caboxy-methyl cellulose (CMC) (1:100w/w), (Niranjana *et al.*, 2009). The talc formulations were packed in polythene bags, sealed and stored at 4℃ until use.

To obtain a final working concentration, with a spore concentration of 1.0×10^8 spores/g, the formula of Goettel and Inglis (1997), N/V×D was used, where, N is the number of conidia, V is the volume of the chamber (constant of 2.5×10^5) and D is the dilution factor.

A given amount (depending on the spore concentration) of each formulated powder was diluted with water and mixed appropriately. The mixture was filtered through a two layered cheese cloth and a drop of 0.05% Tween 20 was added to the filtrate and applied as foliar spray (Fulano *et al.,* 2015). For the plant extracts, 20 millilitres of each aqueous concentrated supernatant of the plant materials were added into five litres of water having one drop of 0.05% Tween 20 and used as foliar sprays (Fulano *et al.*, 2015).

3.4.5 Treatment application

The treatments included three most active selected fungal antagonists at a spore concentration of 1.0×10^8 spores/ml and three crude plant extracts, at 4ml/L the standard check/positive control was Master® 72WP (Mancozeb 64%+Metalaxyl 8%) at 2.5g/L and a negative control (Water). Treatment application begun on foliage immediately on appearance of the first late blight symptoms at the vegetative stage, followed by five subsequent repetitions at seven-day intervals (Mosota *et al.*, 2017). Treatments were applied early in the morning. The study was conducted in a field naturally infested with *P. infestans* inocula.

3.4.6 Assessment of late blight incidence and severity on potatoes

The disease severity and incidence were recorded on a 7-day interval after the appearance of the first blight symptoms in experimental site. Percentage infection (PI) was calculated using the formula by Mosota *et al.* (2017):

$$PI = \frac{N_I}{N_T} \times 100$$

Where: PI is percentage incidence, N_I is Number of infected plants in a plot, and N_T is the total number of plants in a plot.

Disease index (DI) was assessed by randomly tagging 15 infected plants on each plot. The severity of blight was recorded on a 7-day interval on the basis of percentage leaf damaged. Disease severity assessment was graded following a modified 0 to 5 scale from Babu, (1994), where, 0=no infection; 1=Very mild (1 to 10%); 2=Mild (11 to 25%); 3=Moderate (26 to 50%); 4=Severe (50 to 75%); and 5=Very severe (>75%). The percentage disease index (PDI) was calculated using a modified formula from Mckinney (1923) as used by Ramjegathesh *et al.* (2011):

$$PDI = \frac{S_N}{L_T} \times \frac{100}{DG_{MAX}}$$

Where: $\boldsymbol{PDI}$ is percent disease index, $\boldsymbol{S_N}$ is Sum of all numerical ratings, $\boldsymbol{L_T}$ is Total number of leaves observed and $\boldsymbol{DG_{MAX}}$ is Maximum disease grade in the score chart.

3.4.7 Area under the disease progress curve (AUDPC)

The area under the disease progress curve (AUDPC) was computed using Wilcoxson *et al.* (1975) formula as modified by Meena *et al.* (2011).

$$\boldsymbol{AUDPC} = \sum_{\boldsymbol{i=1}}^{\boldsymbol{k}} \frac{\boldsymbol{1}}{\boldsymbol{2}}\ \boldsymbol{1}(\boldsymbol{y_i} + \boldsymbol{y_{i-1}})\ \times \boldsymbol{d}$$

Where $\boldsymbol{AUDPC}$ is area under the disease progress curve, $\boldsymbol{y_i}$ is the disease incidence at ***i*th** day of evaluation, $\boldsymbol{k}$ is the number of successive evaluations and $\boldsymbol{d}$ is the interval between ***i*** and ***i*-1** evaluation of the disease.

3.4.8 Yield assessment

Tubers were harvested per plot and subsequently weighed and the readings were taken in kilograms, this information was then converted to Kilograms per hectare. The harvested tubers were sorted by separating the infected tubers from the healthy tubers and classified according to three categories depending on the tuber diameter namely. These were; ware>60mm diameter, seed 28-60mm (seed size 1: 28-45mm, seed size 2: 45-60mm) diameter and chatt<28mm diameter. Ware and seed categories were classified as marketable, while chatts and those destroyed by pest, diseases and manual injury were classified as non-marketable. Chatts are also used as animal feed (De Haan *et al.*, 2014).

The yield in each plot was recorded and percent yield increase over untreated control was determined using the formula used by Verma *et al.* (2018):

$$\%Y/C = \frac{Tt - Yc}{Yc} \times 100$$

Where, ***%Y/C*** = Percentage yield over the control; ***Yt*** = Yield in treatment; ***Yc*** = yield in control

3.5 Data analysis

The primary survey data on socio-demographic characteristics, potato production practices and potato diseases management practices were analyzed using Statistical Package for Social Sciences (SPSS) software version 20 for descriptive statistics, frequencies and percentages. Laboratory and field data were analyzed using SAS software version 9.2. Analysis of variance (ANOVA) was carried out on percentage growth inhibition (PGI), percentage growth suppression (PGS), and on field data on disease incidence, severity and yield. Mean separations were carried out using Fisher's protected Least Significant Difference (LSD) test ($P \leq 0.05$). The results were presented in percentages descriptively presented using tables, bar graphs, and pie charts.

CHAPTER FOUR: RESULTS

4.1 Factors influencing the prevalence of potato late blight in Nyandarua County

4.1.1 Socio-demographic characteristics of potato farmers in Nyandarua County

There was a difference in gender participation among the respondents where the men dominated potato production accounting for 62% and women at 38%. The age categories were clustered in to nine categories between <20 years and >56 years. Majority of the respondents involved in potato production, about 19% were between 20 to 25 years of age followed by those between 31 to 35 years accounting for 16.2% of the total respondents (Table 4.1).

In relation to level of education, 5.7% of the respondents had not attended to school, of which 4.7% were men and 1.0% women. Majority of the respondents, 43.8% had attained primary level; secondary 26.7%; post-secondary training 23.8% (Table 4.1).

Table 4.1 Demographic characteristic (%) of small-scale potato farmers in Nyandarua County

Variables		Gender frequency (%)	
		Men	Women
Age category (Years)	<20	1.9	-
	20-25	10.4	8.6
	26-30	7.6	6.7
	31-35	9.5	6.7
	36-40	7.6	3.8
	41-45	10.5	3.8
	46-50	7.6	2.9
	51-55	1.9	4.8
	>56	4.7	1.0
Education level	None	1.0	4.7
	Primary	27.6	16.2
	Secondary	18.1	8.6
	Post-secondary	15.2	8.6

Majority of the respondents owned farms (86.4%) while 38.1% farmed on hired land (Table 4.2). Of the respondents who owned land, 28.6% had less than 1 acre; 38.1% = 1-3 acres; 17.1% = 4-6 acres and 6.7% had 7-9 acres. Only 2.9% owned more than 10 acres (Table 4.2).

In regard to area allocated to potato, 47.6% of the respondents allocated area less than 1 acre, closely followed by 1-3 acres at 43.8% and the least being 4-6 acres at 8.6%. Majority of the respondents, 67.6% cultivated potatoes for three seasons in a year. About 29.5% of the respondents cultivated potatoes for only two seasons while those who cultivated potatoes for just a season in a year were 2.9% (Table 4.2).

As regards potato cultivation experience, 34.3% of the respondents responded to have been growing potatoes for over five years. This was followed by 33.3% who had an experience of 3-5 years. Others, 20% had a history of up to two years. Those that had produced potato for only a year were 12.4% (Table 4.2).

Table 4.2 Land tenure (%) and use (%) by small scale potato farmers Nyandarua County

Variables	**Area (acres)**	**Gender frequency (%)**	
		Men (M)	**Women (W)**
Size of owned farm	<1	16.2	12.4
	1-3	21.9	16.2
	4-6	10.5	6.7
	7-9	3.8	2.9
	>10	2.9	-
Area under potato	<1	29.5	17.1
	1-3	24.8	19.0
	4-6	6.7	1.9
Variables	**No. of seasons**	**Men (M)**	**Women (W)**
Planting seasons	One	2.9	-
	Two	18.1	11.4
	Three	41.0	26.7
Potato cultivation experience	One year	8.6	3.8
	Two years	13.3	6.7
	3 - 5 years	19.0	14.3
	>5years	21.0	13.3

4.1.2 Potato production systems in Nyandarua County

Labour utilised by small scale potato farmers in Nyandarua County is either hired or family labour. Farm labour for production of Irish potato is largely hired by

94.3% of the respondents whereas 70.5% make use of the labour from their respective families.

All the respondents reported to be growing Shangi potato variety whereas Asante is only grown by 2.9%. Shangi variety is preferred majorly due to its ready market and high yield. Other reasons for Shangi's preference are early maturity and availability of planting materials.

Table 4.3 Reasons for varietal preference by potato farmers in Nyandarua County

Attributes	Respondents (%)
Shangi variety	
Ready market	86.7
High yield	69.5
Readily available	55.2
Early maturity	57.1
Longer dormancy	23.8
Asante variety	
High yield	2.9
Ready market	2.9
Readily available	1
Early maturity	2.9

The main source of potato seeds by 74.3% of respondents in Nyandarua County is from farmer's previous harvests (Figure 4.1). About 23.8% of the respondents' sources of seeds are from neighbours, and about 14.3% source seeds from certified seed dealers such as KALRO and Agricultural Development Corporation (ADC).

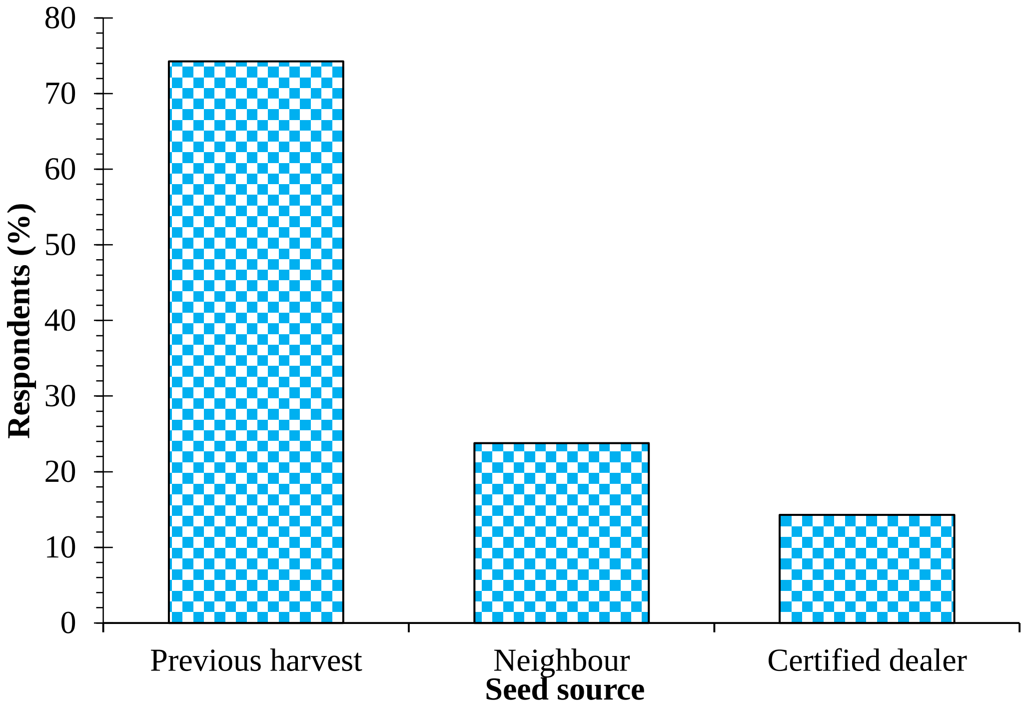


Figure 4.1 Sources of potato tuber seeds by small scale potato farmers in Nyandarua County

Potato is the major cash and food crop grown in Nyandarua County. Some of the crops either inter-cropped or rotated with potato include; cabbages, beans, peas, maize, groundnuts, carrots and spinach. The most practised cropping system is crop rotation at 56.2% of the total respondents closely followed by mono-cropping accounting for 48.6%. Inter cropping is practised by 33.3% of the respondents while the least practised is relay cropping constituting of only 1% (Figure 4.2). Relay cropping is a method of multiple cropping where one crop is seeded into standing second crop well before harvesting of second crop (Tanveer *et al.*, 2017). Combinations of cropping systems are done by 30.5% of the farmers.

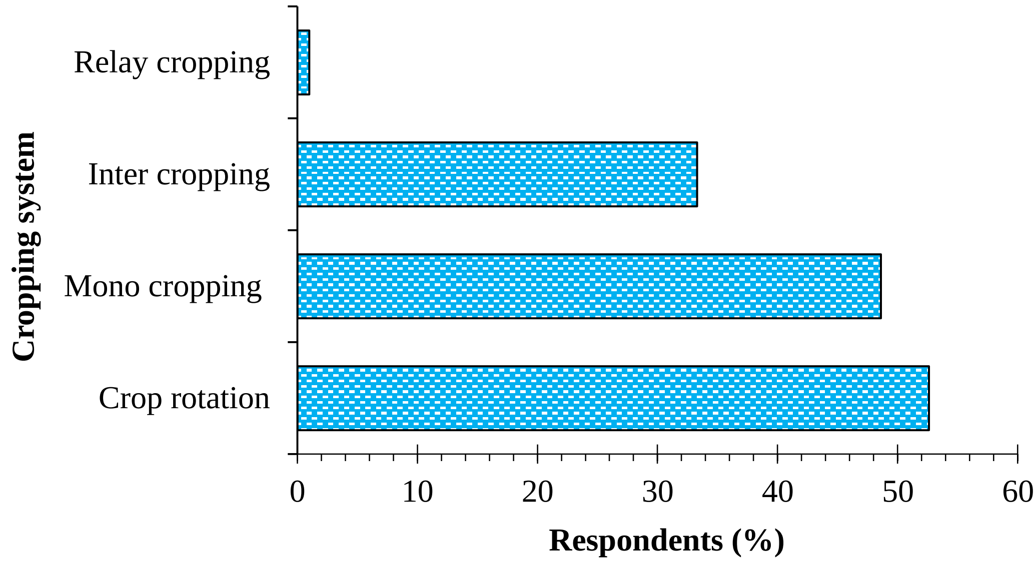


Figure 4.2 Cropping systems practiced by small scale potato farmers in Nyandarua County

Majority of respondents 67.6% in Nyandarua County practise rain fed farming. Surface irrigation is practised by about 32.4% of the farmers who divert irrigation water from dams and rivers.

4.1.3 Major diseases encountered by farmers during potato production and management of late blight in Nyandarua County

With reference to photocards with signs and symptoms of various potato diseases, the major diseases reported to be affecting potato production in Nyandarua County are late blight and early blight. Late blight and bacterial wilt were reported by all the respondents while early blight was reported by 84.8% of the respondents. Viruses were encountered by 43.8% of farmers, dry rot by 13.3% and the least reported disease was potato wart and common scab both at 6.7% (Figure 4.3; Table 4.5).

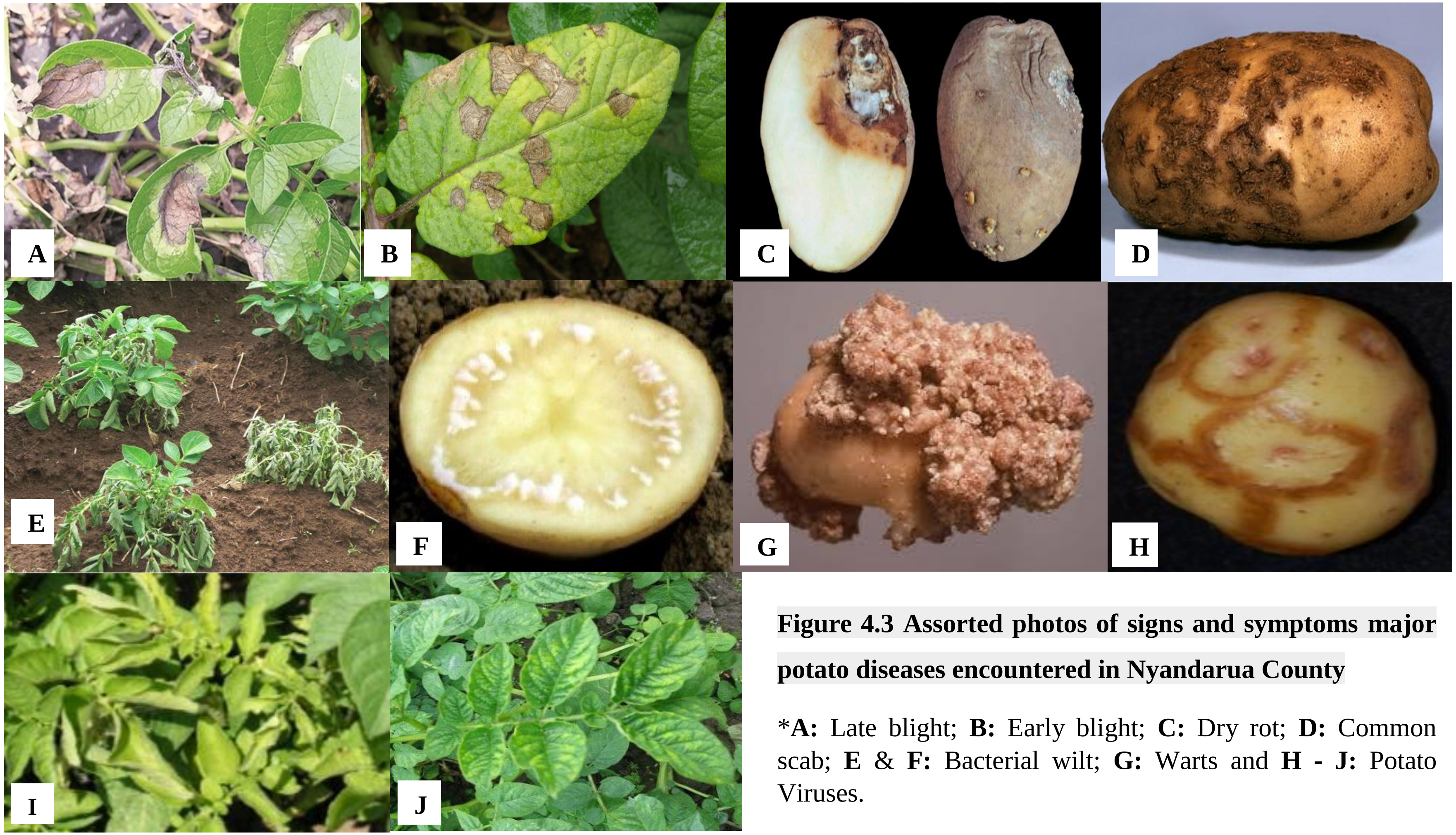


Figure 4.3 Assorted photos of signs and symptoms major potato diseases encountered in Nyandarua County

***A:** Late blight; **B:** Early blight; **C:** Dry rot; **D:** Common scab; **E** & **F:** Bacterial wilt; **G:** Warts and **H - J:** Potato Viruses.

Table 4.4 Major diseases encountered by small scale potato farmers in Nyandarua County

Disease(s)	Respondents (%)
Late blight	100
Bacterial wilt	100
Early blight	84.8
Viruses	43.8
Dry rot	13.3
Potato warts and common scab	6.7

Fungicide application is the only management option the respondents use in managing potato late blight in Nyandarua County. Alternation of fungicides is practiced by about 34.2% of farmers whereas the majority, 65.8% applied fungicides singly. Mistress® 72WP and Ridomil Gold® MZ 68WG are the most widely used fungicides by 40% and 32% of the respondents, respectively to manage potato late blight. Tata Master® 72WP is used by 15.2% of the respondents while Agromax® MZ 720WP by 13.3%. The detailed list of fungicides used to manage potato late blight by the farmers in Nyandarua County is given in Table 4.6 below.

Table 4.5 Fungicides used by small scale potato farmers in management of potato late blight in Nyandarua County

Fungicide	Active ingredients	Agent/Manufacturer	Respondents (%)
Mistress® 72WP	Cymoxanil 8% + Mancozeb 64%	Osho Chemical Industries Ltd.	40
Ridomil Gold® MZ 68WG	Mancozeb 64% + Metalaxyl 4%	Syngenta E.A. Ltd.	32.4
Tata Master® 72WP	Mancozeb 64% + Matalaxyl 8%	Osho Chemical Industries Ltd.	15.2
Agromax® MZ 720WP	Mancozeb 64% + Cymoxanil 8%	Agrosolutions (K) Ltd.	13.3
Sphinx Extra® 713 WDG	Folpet 600g/kg + Dimethomorph 113g/kg	Amiran (K) Ltd.	3.8
Vanguard® 525WDG	Cymoxanil 300 g/kg + Famoxadone 225 g/kg	Kenagro Suppliers Ltd	2.9
Fortress Gold® 72WP	Mancozeb 640g/Kg + Cymoxanil 80g/Kg	Greenlife Crop Protection Africa Ltd	2.9
Tajiri® 72WP	Mancozeb 64% + Cymoxanil 8%	Kenagro Supplies Ltd.	2.9
Twigalaxyl® 72WP	Mancozeb 640g/Kg+ Metalaxyl 80g/Kg	Twiga Chemical Industries Ltd	2.9

Fungicide application regimes against potato late blight disease vary during the wet, long rains seasons (March to May) and dry, short rains seasons (October to December). It was noted that fungicides are applied more during the wet season due to potato late blight's high prevalence. All through the wet season, 42.9% of potato farmers apply fungicides once a week. About 21% apply fungicides twice a week while 3.8% of the respondents apply on a thrice a week regime. In the course of the dry season, 26.7% of farmers apply fungicides on a twice a month whereas 5.7%

apply on a thrice a month regime since the establishment and development of late blight disease is not favoured by the weather conditions (Figure 4.4).

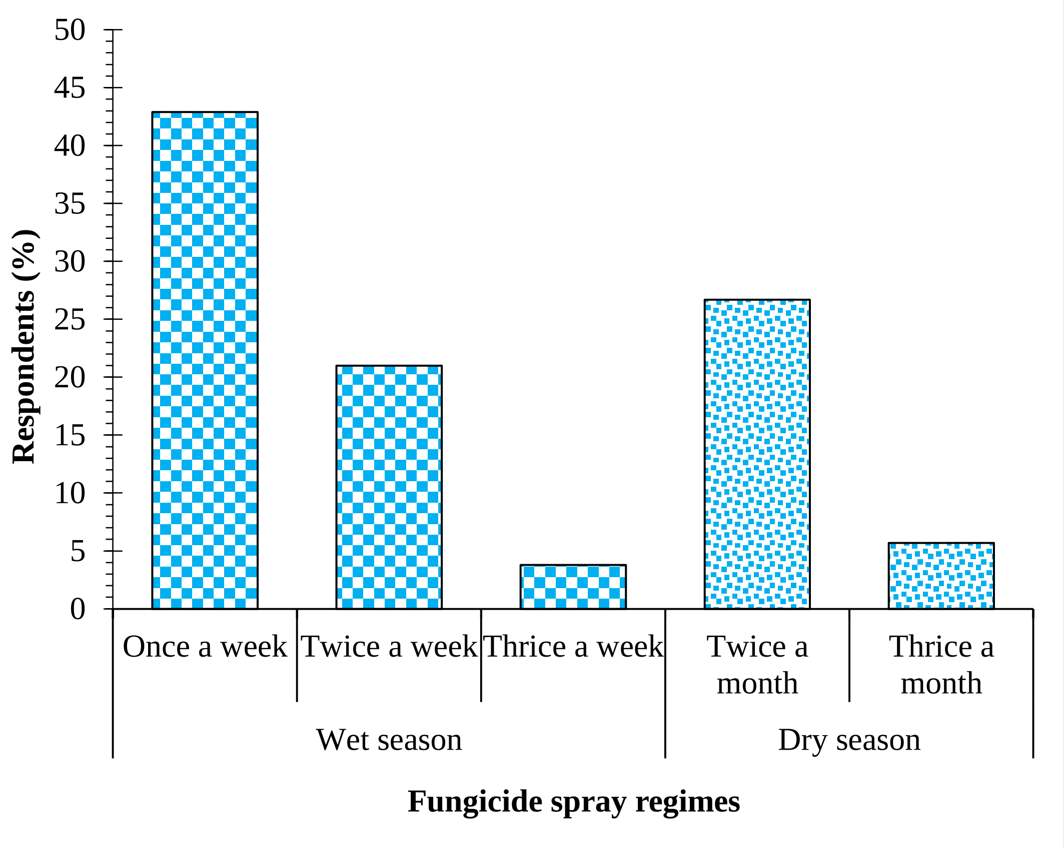


Figure 4.4 Fungicide application regimes for control of *P. infestans* on potato by small scale potato farmers in Nyandarua County

Awareness of biological control by farmers in Nyandarua County is low at 33.3%, though this is slightly higher than the awareness of integrated disease management (IDM) strategy which is at 28.6%. None of the respondents reported to have used any biological product in managing potato late blight despite a botanical BIO CURE F 1.5 WP (*Trichoderma viride* strain TV-1 1.5×10^6 cfu/g) having been registered in Kenya by the Pest Control Products Board (PCPB) for management of *Phytophthora* sp. on a number of horticultural crops.

4.1.4 Potato yield and post-harvest practices in Nyandarua County

Majority of the respondents 27.6% attain an average yield of 16 to 20 bags per acre which are packaged at 110kg per bag by the brokers. Yield of 21 to 25 bags were achieved by 26.7% followed by 11 to 15 bags per acre which was realised 20% of respondents. Whereas some farmers harvest more than 26 bags per acre, the least attainable yield was less than 5 bags per acre (Figure 4.5).

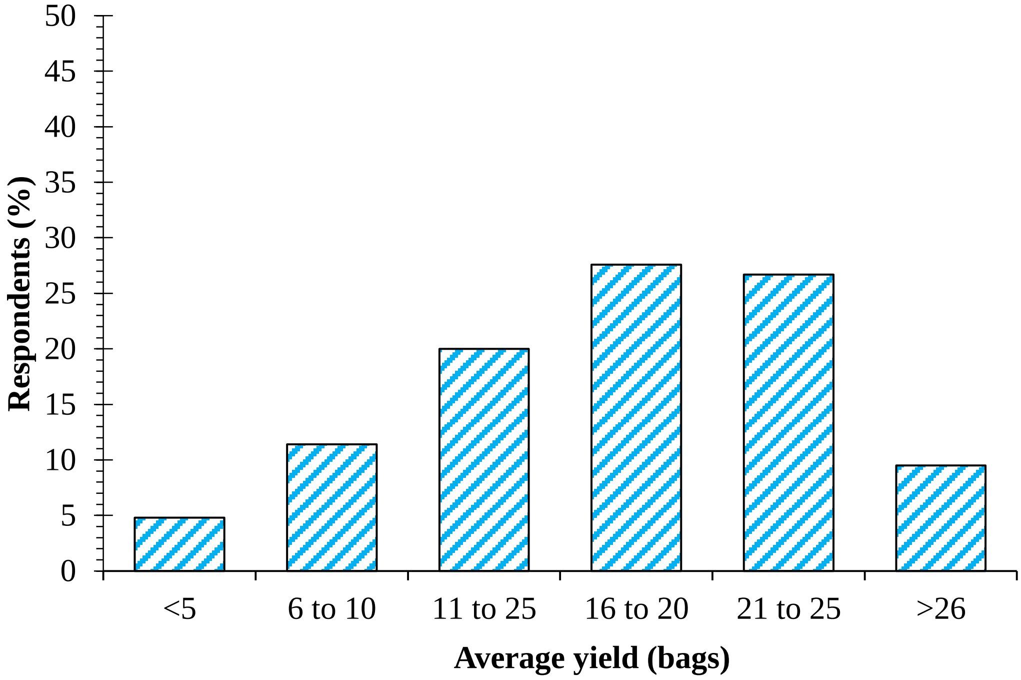


Figure 4.5 Average Potato Yield (Bags per acre) attained by small scale potato farmers in Nyandarua County

*1 bag = 110kg

After harvesting, majority of the respondents, 82.9% use the plant debris from potato fields for animal feed. Some respondents, about 29.5% bury to decompose while 13.3% leave the debris to dry in the open field.

4.2 Antifungal activity of antagonistic fungi against *Phytophthora infestans* under laboratory conditions

4.2.1 *Phytophthora infestans* (Mont.) de Bary

Phytophthora infestans was examined under compound microscope (XSZ-107T). Some of the observable characteristics were white hyaline mycelia and much branched, coenocytic hyphae, branched sporangiophores and lemon-shaped sporangia with papilla/pedicel and semi-papilla (Plate 4.1).

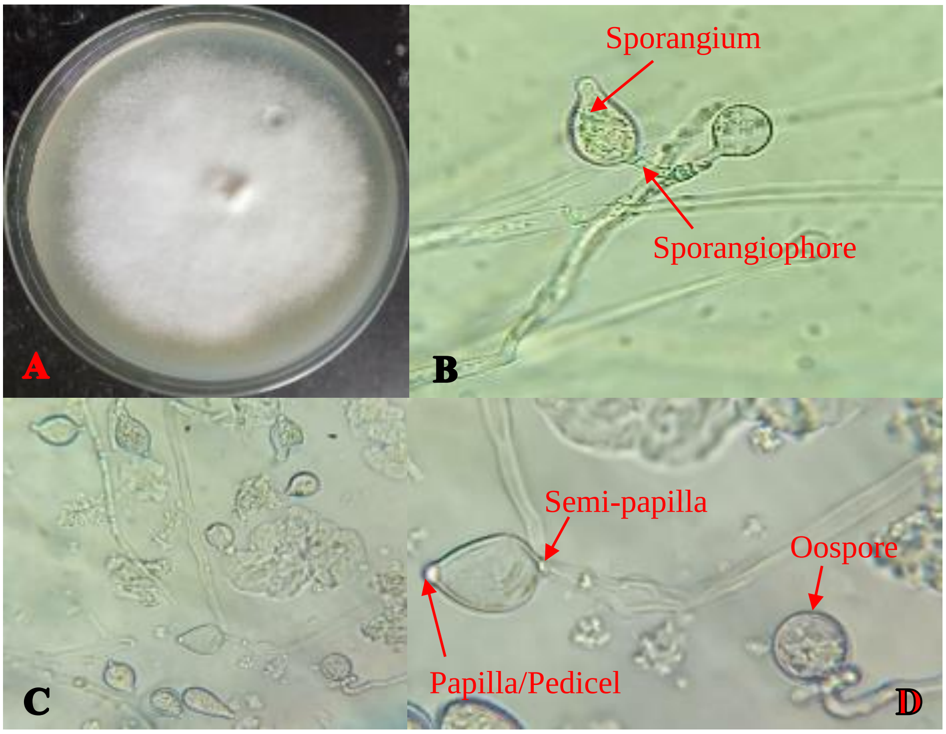


Plate 4.1 ***Phytophthora infestans***

***A** = *Phytophthora infestans* mycelia on pea Agar medium, **B, C, D** = Sporangiophores and lemon-shaped sporangia

4.2.2 *In vitro* activity of fungi against *Phytophthora infestans*

A total of 46 fungi from 9 genera were isolated from the soil collected from Nyandarua, Murang'a, Embu and Meru Counties (Table 4.7).

Table 4.6 Fungal genera isolated from soil samples collected form Nyandarua, Murang'a, Embu and Meru Counties

Fungal genera	Frequency
Trichoderma spp.	22
Fusarium spp.	6
Aspergillus spp.	4
Rhizopus spp.	2
Cheatomium spp.	3
Rhizoctonia sp.	1
Penicillium spp.	4
Paecilliomyces spp.	3
Verticillium sp.	1
Total	**46**

The fungi were isolated and evaluated *in vitro* for efficacy against *P. infestans*. The *in vitro* study was conducted using ten most active fungi in terms of growth and colonisation to test for their inhibitory effect on *P. infestans*. By the tenth DAI, all the evaluated isolates *in vitro* caused varying significant (F=24.18; df=10, 32; P<0.001) inhibitory effect on *P. infestans* mycelia compared to the water control treatment (Table 4.8). The fungal isolates caused varying inhibitory effect over the treatment period where the highest results were obtained on the tenth day after inoculation.

Trichoderma hamatum exhibited the highest inhibitory effect over all the other evaluated fungi by the tenth day after inoculation achieving 50.2% growth inhibition. *Trichoderma hamatum*, *T. afroharzianum* and *T. asperellum* caused inhibitions of 50.2, 48.5 and 47.8%, respectively, and were the three most active fungal isolates (Table 4.8). These three isolates, *T. hamatum*, *T. afroharzianum* and *T. asperellum* were therefore considered for further evaluation under field condition. *Trichoderma* sp. 2, 3 and 6 caused a significant ($F=24.18$; df = 3, 11; $P<0.001$) inhibitory activity across the days of evaluation, although at lower inhibitory percentages compared to *Trichoderma hamatum*, *T. afroharzianum* and *T. asperellum*.

Table 4.7 Mean growth inhibitions of *P. infestans* mycelia by fungi *in vitro*

Isolate	Days after inoculation (DAI) (Mean±S.E)				LSD	P-value	F-value	CV (%)
	4	6	8	10				
Trichoderma hamatum	40.9±3.8^{aA}	43.6±4.1^{aA}	48.5±4.6^{aA}	50.2±3.2^{aA}	12.86	0.371	1.20	14.9
Trichoderma afroharzianum	43.3±2.6^{aA}	43.6±2.7^{aA}	46.4±2.9^{abA}	48.5±3.4^{aA}	9.47	0.564	0.73	11.1
Trichoderma asperellum	41.6±4.4^{aA}	41.6±2.6^{aA}	43.9±2.5^{abA}	47.8±2.7^{aA}	10.00	0.475	0.92	12.1
Trichoderma sp. 1	40.3±8.5^{aA}	40.7±9.0^{aA}	45.4±7.9^{abA}	47.4±7.5^{aA}	26.95	0.908	0.18	33.0
Trichoderma sp. 2	40.7±0.5^{aC}	40.9±0.5^{aC}	42.4±0.4^{abB}	46.3±0.4^{aA}	1.44	< 0.001	34.80	1.8
Trichoderma sp. 3	35.5±2.2^{aC}	40.4±0.5^{aB}	41.2±0.4^{abB}	45.5±0.4^{aA}	3.74	0.002	12.88	4.9
Trichoderma sp. 4	37.5±1.8^{aB}	38.7±1.5^{aB}	41.1±1.9^{abAB}	45.0±1.6^{aA}	5.55	0.058	3.80	7.3
Trichoderma sp. 5	38.7±2.1^{aAB}	37.7±2.2^{aB}	41.3±2.1^{abAB}	44.6±1.6^{aA}	6.45	0.142	2.41	8.5
Trichoderma sp. 6	38.4±1.6^{aB}	38.6±0.8^{aB}	40.2±0.4^{abB}	44.3±0.4^{aA}	3.04	0.007	8.42	4.0
Trichoderma sp. 7	38.9±0.5^{aAB}	37.7±2.5^{aB}	38.8±2.3^{bAB}	44.7±1.4^{aA}	6.11	0.108	2.81	8.1
Control	0^{bA}	0^{bA}	0^{cA}	0^{bA}	-	-	-	-
LSD (<0.001)	9.96	9.97	9.38	8.42	-	-	-	-
P-value	< 0.001	< 0.001	< 0.001	< 0.001	-	-	-	-
F-value	12.73	13.19	17.19	24.18	-	-	-	-
CV (%)	16.4	16.1	14.2	11.8	-	-	-	-

Means accompanied by the same letter(s) in each row (uppercase) and column (lowercase) are not significantly different at P≤0.001. Fisher's Least Significant Difference test.

4.3 *In vitro* activity of plants with bioactive potential against *P. infestans in vitro*

By the tenth DAI, plant extracts caused varying significant (F=11.67, 11.67; df=11, 59; P<0.001) suppressive effect on *P. infestans* mycelia compared to the untreated control. *Azadirachta indica, P. lentiscus* and *T. diversifolia,* caused 59.1, 52.2 and 50.3% mycelial suppression, respectively. The extracts of *A. indica, P. lentiscus* and *T. diversifolia* were the most active against *P. infestans in vitro* among the evaluated extracts, and therefore they were considered for further screening under field condition. *Azadirachta indica, T. diversifolia, X. strumarium, L. camara, C. citritus* and *A. aspera* showed a significant (df=3, 19; P<0.05) suppressive activity across the days from the 4th day after inoculation to the 10th day. *Callistemon citrinus* showed the least suppressive activity ten DAI with 27.5% in the *in vitro* evaluations (Table 4.9).

Table 4.8 Mean growth suppressions of *P. infestans* mycelia by plant extracts *in vitro*

Source of extracts		Days after inoculation (DAI) (Mean±S.E)							
Common name	Scientific name	4	6	8	10	LSD	P-Value	F-value	CV (%)
Neem	*Azadirachta indica*	49.0 ± 1.7^{aB}	53.9 ± 1.4^{aAB}	55.2 ± 1.8^{aA}	59.1 ± 2.3^{Aa}	5.41	0.010	5.37	7.4
Mastic	*Pistacia lentiscus*	43.9 ± 2.6^{aB}	46.8 ± 2.8^{abAB}	48.4 ± 2.1^{abAB}	52.2 ± 1.8^{abA}	7.11	0.138	2.12	11.1
Mexican sunflower	*Tithonia diversifolia*	40.7 ± 0.5^{abC}	47.7 ± 1.7^{abAB}	45.3 ± 1.3^{abcB}	50.3 ± 2.0^{abA}	4.35	0.002	7.86	7.1
Mexican marigold	*Tagetes minuta*	39.1 ± 10.2^{abA}	44.0 ± 11.6^{abcA}	44.7 ± 11.8^{abcA}	47.9 ± 12.1^{abcdA}	34.43	0.958	0.10	58.5
Sodom apple	*Solanum incanum*	40.0 ± 1.4^{abA}	36.6 ± 3.0^{bcdeA}	36.4 ± 3.3^{bcdeA}	43.9 ± 2.3^{bcdeA}	7.83	0.190	1.79	14.9
European cocklebur	*Xanthium strumarium*	25.8 ± 1.2^{cC}	40.1 ± 2.6^{bcdB}	42.5 ± 2.9^{abcdAB}	48.2 ± 2.2^{abcA}	6.70	< 0.001	18.08	12.7
Jimson weed	*Datura stramonium*	30.0 ± 2.5^{bcA}	35.8 ± 1.4^{bcdeA}	35.2 ± 2.0^{cdeA}	36.1 ± 2.4^{cdefA}	6.37	0.180	1.85	13.9
Lantana	*Lantana camara*	26.3 ± 1.5^{cB}	32.7 ± 1.5^{cdeA}	35.4 ± 1.7^{cdeA}	34.5 ± 1.9^{efA}	4.88	0.005	6.37	11.3
Lemon grass	*Cymbogopon citratus*	23.7 ± 3.1^{cB}	30.3 ± 3.4^{deAB}	33.1 ± 2.8^{cdeA}	36.0 ± 2.3^{cdefA}	8.77	0.050	3.25	21.2
Devils horsewhip	*Achiranthes aspera*	23.1 ± 1.0^{cB}	27.8 ± 2.2^{deB}	29.9 ± 2.8^{deAB}	35.1 ± 2.9^{defA}	7.00	0.018	4.53	18.1
Lemon bottlebrush	*Callistemon citrinus*	24.3 ± 6.6^{cA}	26.5 ± 6.7^{eA}	28.7 ± 7.2^{eA}	27.5 ± 7.1^{fA}	20.73	0.973	0.07	57.8
Control	-	0^{dA}	0^{fA}	0^{fA}	0^{gA}	-	-	-	-
LSD (<0.0001)	-	11.04	12.403	12.8	12.786	-	-	-	-
P-value	-	< 0.001	< 0.001	< 0.001	< 0.001	-	-	-	-
F-value	-	11.44	10.25	9.51	11.67	-	-	-	-
CV (%)	-	28.5	27.7	27.8	25.6	-	-	-	-

Means followed by the different letter(s) in each row (uppercase) and column (lowercase) are significantly different at P≥0.001. Fisher's Least Significant Difference test.

4.4 Physicochemical characteristics of the soil at the experimental site in KALRO-Tigoni Horticultural center

The interpretation and recommendations of the results from the ccomposite soil samples from the field study site taken for physiochemical characteristics analysis at the National Agricultural Research Laboratories (NARL), Kabete were as follows: The soil texture is sandy clay. Based on the soil fertility results, the soil was found to be acidic for plants growth, hence acidic fertilizers such as DAP and urea should be avoided, but rather lime should be applied at 400t/ha four weeks before planting to raise the soil pH. The soil was found to be calcium deficient. Just before planting, 2 tons/acre of well decomposed manure or compost should be applied to improve the soil organic matter and 80kg/acre of N:P:K 23:23:0 should be applied, ensuring the manure and the fertilizer are well worked to avoid close/direct contact with seeds (Table 4.10).

Table 4.9 Physicochemical properties of soils from KALRO-Tigoni, Kiambu County

Field	TIGONI 1		TIGONI 3	
Soil depth (10-15cm)	**Top**		**Top**	
Soil Fertility results	**Value**	**Class**	**Value**	**Class**
Soil pH	4.74	SA	4.63	SA
Exch. Acidity me%	0.8	A	0.9	H
Total Nitrogen %	0.25	A	0.25	A
Total Organic Carbon %	2.58	A	2.62	A
Phosphorus ppm	40	A	30	A
Potassium me%	0.78	A	1.34	A
Calcium me%	1.2	Lo	1.8	Lo
Magnesium me%	1.03	A	1.00	A
Manganese me%	1.46	A	1.33	A
Copper ppm	0.71	A	1.26	A
Iron ppm	44.4	A	33.6	A
Zinc ppm	37.9	A	21.4	A
Sodium me%	0.24	A	0.22	A
Soil Texture	**SC**		**SC**	
	48%	Sand		48%
	40%	Clay		40%
	12%	Silt		12%
Bulk Density (g/cm^3)	1.34		1.33	
Particle Density (g/cm^3)	2.51		2.55	
Porosity (%)	46.61		47.84	

****TIGONI 1** = May to August 2018; **TIGONI 3** = April to July 2019 site; **SC** = Sandy Clay; **SA** = Strong acid; **H** = High; **A** = Adequate; **Lo** = Low; **ppm** = parts per million **me** = milliequivalents.

4.5 On field activity of fungal antagonists and crude plant extracts against potato late blight in Kiambu County

4.5.1 Late blight incidence on Shangi and Tigoni varieties

Late blight was present at varying levels throughout the first and third seasons on Shangi (Table 4.11 and 4.13) and Tigoni variety (Table 4.12 and 4.14). Disease incidence was generally lower in season one (May to August 2018) compared to season three (April to July 2019) due to unfavourable weather conditions; low precipitation, low relative humidity and high temperatures, during the vegetative stage (Figure 4.6). This persistent disease incidence was attributed to the highly favourable weather conditions present throughout May to August 2018 and April to July 2019 (Figure 4.6). No disease incidence was recorded during the second season (December 2018 to March 2019) due to the prevailing unfavourable weather conditions i.e., relatively high temperatures (Figure 4.6), low relative humidity (Figure 4.6) and precipitation (Figure 4.6), for the establishment of late blight. During May to August 2018, statistically significant ($F=271.4$; $df=4, 31$; $P<0.05$) disease incidence levels occurred from week 9 all through to week 12 on the two potato varieties whereas during April to July 2019, significant ($F=216.08$; $df=4, 31$; $P<0.05$) incidence levels occurred from week 10 through to week 12 on both Shangi and Tigoni varieties.

Throughout May to August 2018, there was a drastic decrease on the disease incidence levels on both varieties (Table 4.11 and 4.12). treated with fungal isolates and plant extracts on the 9th week after planting (2nd week of data collection) due to the sudden rise in temperatures (Figure 4.6). This was followed by a steady rise of incidence levels to week 12 after planting (5th week of data collection) caused by the steady increase in temperature (Figure 4.6). On the other hand, disease incidence levels on both varieties treated with fungal isolates and plant extracts rose gradually all through from week 8 to week 12 during April to July 2019 (Table 4.13 and 4.14). Significant ($F=271.4$; $df=4, 31$; $P<0.05$) rise in disease incidence levels was caused by water treatment (control) on both Shangi and Tigoni varieties throughout May to August 2018 and April to July 2019. Mistress® generally caused steady disease decline on both varieties and seasons.

Table 4.10 Mean disease incidence for late blight assessed on Shangi potato variety treated with plant extracts and antagonistic fungi between May and August 2018 (Season one)

Treatments	Weeks after planting (Mean±S.E)					LSD	P-value	F-value
	8	9	10	11	12			
Trichoderma hamatum	96.1±2.38^{aA}	57.7±8.25^{cB}	93.7±2.23^{bA}	98.0±1.18^{aA}	98.7±1.32^{aA}	12.19	< 0.001	18.78
Trichoderma afroharzianum	100±0.0^{aA}	66.0±3.10^{bcB}	99.0±1.0^{abA}	99.1±0.93^{aA}	100.0±0.0^{aA}	4.57	< 0.001	97.98
Trichoderma asperellum	98.5±0.85^{aA}	68.3±1.58^{bcB}	93.7±2.71^{bA}	97.7±2.27^{aA}	98.6±1.47^{aA}	5.70	< 0.001	47.49
Azadirachta indica	97.9±2.14^{aA}	67.7±1.38^{bcB}	99.3±0.68^{aA}	100.0±0.0^{aA}	96.7±2.92^{aA}	4.38	< 0.001	90.85
Pistacia lentiscus	96.7±2.64^{aA}	71.0±2.58^{bB}	98.6a±0.83^{abA}	95.9±2.48^{aA}	99.0±0.0^{aA}	6.26	< 0.001	32.97
Tithonia diversifolia	95.9±2.69^{aA}	65.7±3.59^{bcB}	94.0±3.30^{abA}	96.3±1.42^{aA}	96.3±2.49^{aA}	8.44	< 0.001	23.01
Master®*	94.2±2.56^{aA}	61.0±.99^{bcB}	55.7±5.74^{cB}	40.2±1.52^{bC}	36.0±2.92^{bC}	9.72	< 0.001	51.20
Control (Water)	97.1±1.39^{aA}	92.3±1.99^{aB}	98.4±0.92^{abA}	100.0±0.0^{aA}	100.0±0.0^{aA}	3.35	0.0011	8.12
LSD	5.95	12.14	5.43	4.37	4.98	-	-	
P-value	0.635	< 0.001	< 0.001	< 0.001	< 0.001	-	-	
F-value	0.75	6.30	62.28	188.80	168.04	-	-	

Means accompanied by similar letter(s) in each row (uppercase) and column (lowercase) are not significantly different. Fisher's protected Least Significant Difference test at $P \leq 0.001$. Master®* = Synthetic pesticide.

Table 4.11 Mean disease incidence for late blight assessed on Tigoni potato variety treated with plant extracts and antagonistic fungi between May and August 2018 (Season one)

Treatments	Weeks after planting (Mean±S.E)					LSD	P-value	F-value
	8	9	10	11	12			
Trichoderma hamatum	92.3±1.7^{aA}	44.0±1.81^{bcB}	97.7±2.27^{aA}	98.4±1.56^{aA}	92.7±4.30^{aA}	7.65	< 0.001	82.98
Trichoderma afroharzianum	89.4±2.68^{aB}	45.7±3.42^{bC}	97.7±2.27^{aA}	98.2±1.85^{aA}	100.0±0.0^{aA}	7.06	< 0.001	96.56
Trichoderma asperellum	89.5±7.73^{aA}	46.7±3.81^{bB}	96.7±3.33^{aA}	98.5±1.47^{aA}	95.0±5.0^{aA}	14.30	< 0.001	21.20
Azadirachta indica	90.1±0.98^{aB}	40.0±1.44^{bcdC}	97.7±1.32^{aA}	100.0±0.0^{aA}	95.6±2.64^{aA}	4.62	< 0.001	271.40
Pistacia lentiscus	86.6±4.96^{aB}	37.0±1.26^{cdC}	96.6±1.14^{aA}	96.8±2.01^{aA}	100.0±0.0^{aA}	7.57	< 0.001	110.80
Tithonia diversifolia	91.1±3.24^{aB}	45.3±4.25^{bC}	96.4±2.62^{aAB}	98.9±1.09^{aAB}	100.0±0.0^{aA}	8.17	< 0.001	73.89
Master®*	82.8±2.71^{aA}	35.3d1.59^{dB}	34.7±1.3^{bBC}	27.3±3.96^{bCD}	22.8±0.67^{bD}	7.76	< 0.001	88.49
Control (Water)	87.4±4.02^{aB}	85.0±2.40^{aB}	98.7±1.32^{aA}	100.0±0.0^{aA}	100.0±0.0^{aA}	6.55	3.351	11.54
LSD	11.73	7.96	6.54	5.55	7.36	-	-	-
P-value	0.790	< 0.001	< 0.001	< 0.001	< 0.001	-	-	-
F-value	0.55	33.48	97.10	176.20	111.46	-	-	-

Means accompanied by similar letter(s) in each row (uppercase) and column (lowercase) are not significantly different. Fisher's protected Least Significant Difference test at P ≤ 0.001. Master®* = Synthetic pesticide.

Table 4.12 Mean disease incidence for late blight assessed on Shangi potato variety treated with plant extracts and antagonistic fungi between April and July 2019 (Season three)

Treatments	Weeks after planting (Mean±S.E)					LSD	P-value	F-value
	8	9	10	11	12			
Trichoderma hamatum	65.5±1.02^{abC}	68.0±2.44^{abC}	88.3±2.14^{aB}	91.6±2.17^{bcAB}	95.8±1.66^{bcA}	6.15	< 0.001	51.78
Trichoderma afroharzianum	63.3±2.21^{bcC}	68.3±3.01^{abC}	88.3±0.83^{aB}	90.8±3.01^{bcAB}	95.8±1.67^{bcA}	7.24	< 0.001	39.91
Trichoderma asperellum	60.0±0.00^{cB}	63.3±1.67^{bB}	89.0±2.18^{aA}	88.3±3.33^{cA}	92.5±1.44^{cA}	6.41	< 0.001	58.82
Azadirachta indica	59.2±0.83^{cD}	64.2±0.83^{bC}	89.2±0.83^{aB}	95±1.44^{abA}	96.7±0.83^{abA}	3.11	< 0.001	326.07
Pistacia lentiscus	61.7±0.83^{bcC}	65.8±3.01^{bC}	88.3±1.67^{aB}	93.3±1.67^{bcAB}	96.7±0.83^{abA}	5.63	< 0.001	82.57
Tithonia diversifolia	60.0±2.89^{cB}	65.0±3.82^{bB}	88.2±2.08^{aA}	94.2±2.21^{abcA}	95.8±0.83^{bcA}	8.07	< 0.001	43.50
Master®*	58.9±1.95^{cB}	64.1±1.29^{bA}	58.8±2.37^{bB}	52.6±0.08^{dC}	46.5±0.77^{dD}	4.81	< 0.001	19.59
Control (Water)	69.27±2.07^{aC}	73.4±2.07^{aC}	92.0±1.36^{aB}	100±0.0^{aA}	100±0.0^{aA}	4.55	< 0.001	103.79
LSD	5.16	7.35	5.33	6.23	3.54	-	-	-
P-value	0.006	0.150	< 0.001	< 0.001	< 0.001	-	-	-
F-value	4.45	1.83	36.59	50.58	243.23	-	-	-

Means accompanied by similar letter(s) in each row (uppercase) and column (lowercase) are not significantly different. Fisher's protected Least Significant Difference test at P ≤ 0.001. Master®* = Synthetic pesticide.

Table 4.13 Mean disease incidence for late blight assessed on Tigoni potato variety treated with plant extracts and antagonistic fungi between April and July 2019 (Season three)

Treatments	Weeks after planting (Mean±S.E)					LSD	P-value	F-value
	8	9	10	11	12			
Trichoderma hamatum	56.5±1.79^{cC}	63.5±4.23^{aC}	78.5±1.71^{bcB}	82.3±0.66^{bcB}	91.3±2.03^{bA}	7.53	< 0.001	35.20
Trichoderma afroharzianum	59.6±1.21^{bcD}	67.6±1.81^{aC}	79.0±1.52^{bcB}	87.2±1.29^{bcA}	91.5±0.74^{bA}	4.27	< 0.001	94.64
Trichoderma asperellum	63.6±0.75^{abD}	67.7±2.28^{aD}	75.4±1.75^{cC}	84.7±1.47^{bcB}	92.4±1.37^{bA}	5.05	< 0.001	55.05
Azadirachta indica	59.7±0.34^{bcB}	66.4±2.17^{aB}	79.0±1.52^{bcA}	78.9±7.42^{cA}	89.1±2.33^{bA}	11.58	0.002	9.92
Pistacia lentiscus	60.8±0.83^{abcC}	67.2±1.44^{aC}	80.7±3.16^{bcB}	82.5±1.44^{bcB}	90.0±1.44^{bA}	5.80	< 0.001	49.43
Tithonia diversifolia	64.7±1.73^{aC}	62.5±1.23^{aC}	82.5±1.75^{bB}	88.3±2.14^{abA}	91.6±0.80^{bA}	5.05	< 0.001	58.51
Master®*	61.2±2.53^{abAB}	66.4±2.52^{aA}	58.4±1.12^{dBC}	54.3±2.37^{dC}	46.6±1.19^{cD}	6.46	< 0.001	15.60
Control (Water)	59.5±1.68^{bcD}	68.2±1.09^{aC}	88.5±0.52^{aB}	96.5±1.76^{aA}	100±0.0^{aA}	3.82	< 0.001	216.08
LSD	4.52	6.88	5.32	9.16	4.25	-	-	-
P-value	0.041	0.584	< 0.001	< 0.001	< 0.001	-	-	-
F-value	2.81	0.82	24.06	16.26	145.24	-	-	-

Means accompanied by similar letter(s) in each row (uppercase) and column (lowercase) are not significantly different. Fisher's protected Least Significant Difference test at P ≤ 0.001. Master®* = Synthetic pesticide.

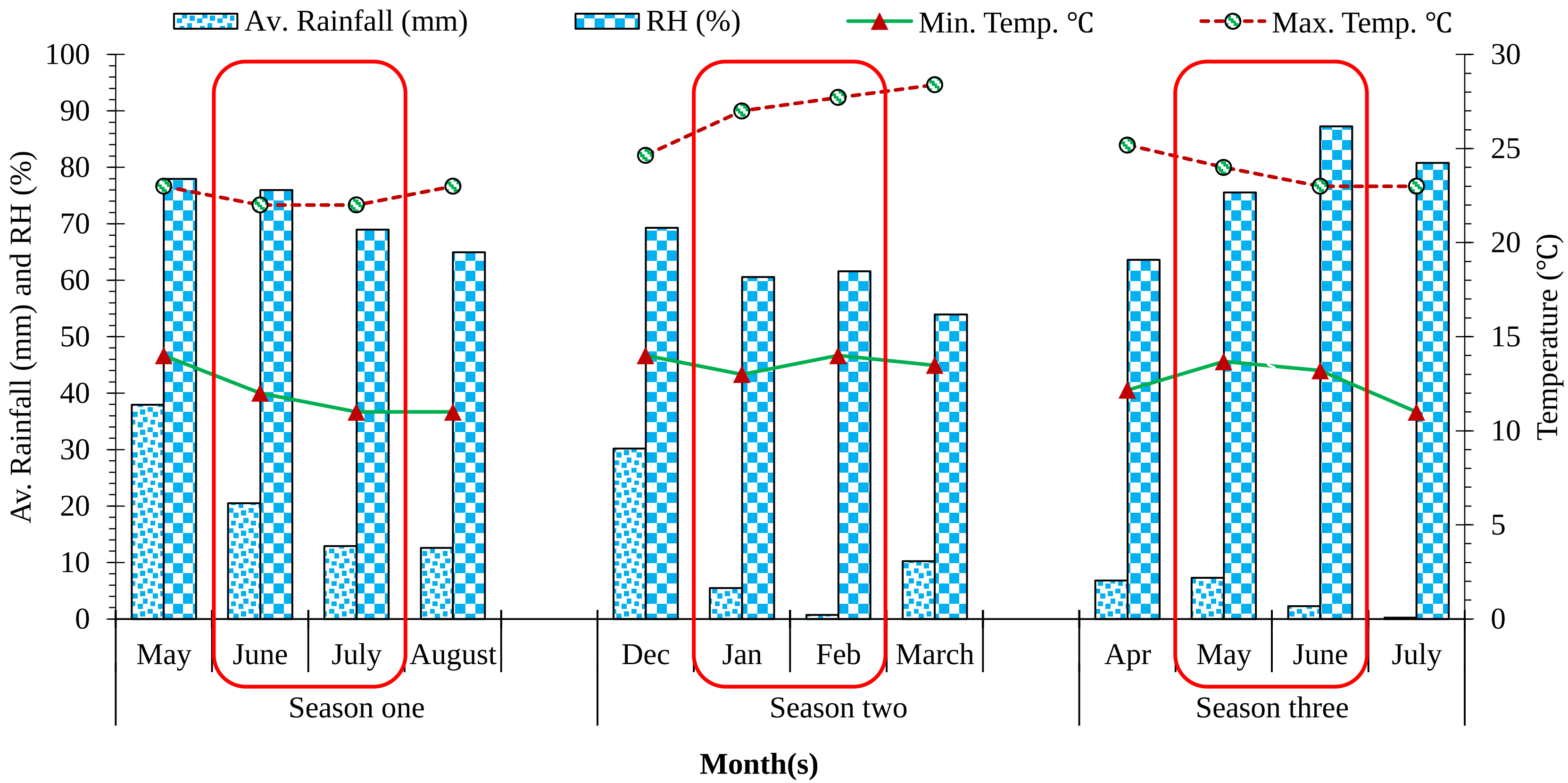


Figure 4.6 Average Rainfall (mm), Relative humidity (%), minimum and maximum temperature (℃) recorded during the three seasons of assessment from May to August 2018 (Season one-long rains), December 2018 to March 2019 (Season two-short rains) and April to July 2019 (Season three-long rains). * Highlighted region covers the vegetative stage.

(Source; KALRO-Tigoni weather station)

4.5.2 Late blight severity on Shangi and Tigoni varieties

Disease incidence was generally lower from May to August 2018 compared to April to July 2019 due to unfavourable weather conditions; low precipitation, low relative humidity and high temperatures, during the vegetative stage (Figure 4.6). No disease incidence was recorded during the second season. Late blight was generally more severe during April to July 2019 (Table 4.17 and 4.18) compared to May to August 2018 (Table 4.15 and 4.16). Disease severity levels were lower on Tigoni variety than on Shangi during both seasons of May and August 2018 and April to July 2019. Disease severity levels varied among the treatments, where some showed significant differences (F=112.92; df=7, 23: 31; P=0.001) when compared to both the positive (Master®) and the negative (water) control.

In the two seasons, late blight intensity gradually increased over time in both varieties upon treatment with the fungal isolates and extracts. Highest severity levels on all the treatments except Master® was recorded on the 12th week after planting (final date of data collection). The antagonistic fungi and plant extracts statistically significantly (F=321.4; df=7, 23: 31; P=0.001) reduced the disease severity when compared to the negative control.

On Shangi variety, May to August 2018, *T. asperellum* had a higher performance in reducing disease severity levels among both the fungal isolates and plant extracts. *Trichoderma asperellum* reduced late blight intensity by 65.7% which was higher than 67.7 and 79.3% intensity for *T. hamatum* and *T. afroharzianum, respectively*. Similarly, *P. lentiscus* causing 71.7% intensity was higher that *T. diversifolia* with 73.3% and *A. indica* with 74% (Table 4.15). During April to July 2019, *Trichoderma asperellum* similarly reduced the intensity of late blight by 83%

which was significantly (P=0.001) higher than 85.9 and 87.4% for *T. afroharzianum* and *T. hamatum*, respectively (Table 4.17).

On Tigoni variety, May to August 2018, *T. hamatum* highly reduced blight intensity among both the fungal isolates and plant extracts. *Trichoderma hamatum* reduced disease severity with an intensity of 54% which was higher than 54.3 and 55% intensities for *T. afroharzianum* and *T. asperellum*, respectively. *Azadirachta indica* with 59.3% intensity reduced late blight intensity higher than that of *P. lentiscus* at 59.7% and *T. diversifolia* 71% intensity (Table 4.16). During April to July 2019, *T. hamatum* with 65.2% disease severity reduction was higher than *T. asperellum* at 65.9% and *T. afroharzianum* with 70.4% in reduction. Among the plant extracts, *A. indica* reduced disease intensity by 66.7% which was higher than 69.6 and 73.3% intensities for *P. lentiscus* and *T. diversifolia,* respectively (Table 4.18).

During season one (May to August 2018), the fungal isolates and plant extracts caused no significant (F=17.69; df = 4, 14; P=0.001) disease reduction on Shangi variety across the weeks of evaluation. On the other hand, on Tigoni variety, all the plant extracts caused a significant (F=35.38; df=4, 14; P=0.001) disease reduction across the weeks of evaluation. During season three (April to July 2019) on both Shangi and Tigoni varieties, all the fungal isolates and plant extracts caused a significant (F=321.40; df=4, 19; P=0.001) disease severity reduction across the weeks of evaluation. In both May to August 2018 and April to July 2019, Tata Master® 72WP (positive control) had the best overall performance in reducing disease severity levels in both varieties evaluated. On the other hand, late blight severity levels were highest on the plants treated with water (negative control) on both varieties over the evaluation period.

Table 4.14 Mean disease severity for late blight assessed on Shangi potato variety treated with plant extracts and antagonistic fungi between May and August 2018 (Season one)

Treatment	Weeks after planting (Mean±S.E)					LSD	P-value	F-value
	8	9	10	11	12			
Trichoderma hamatum	55.0±15.0^{aA}	57.7±8.24^{bA}	60.7±5.06^{bA}	67.7±7.57^{bcA}	67.7±7.27^{bA}	27.91	0.813	0.39
Trichoderma afroharzianum	60.0±8.17^{aB}	66.0±3.10^{abAB}	69.7±1.26^{abAB}	75.7±4.26^{bA}	79.3±6.19^{bA}	15.63	0.120	2.19
Trichoderma asperellum	70.0±5.77^{aAB}	68.3±1.58^{abAB}	60.3±3.83^{bB}	74.3±2.58^{bcA}	65.7±2.13^{bAB}	10.58	0.119	2.19
Azadirachta indica	60.0±8.17^{aB}	67.7±1.37^{abAB}	69.7±1.48^{abAB}	70.3±1.48^{bcAB}	74.0±3.51^{bA}	12.45	0.231	1.58
Pistacia lentiscus	70.0±5.77^{aA}	71.0±2.58^{aA}	63.0±5.64^{bA}	73.7±2.90^{bcA}	71.7±6.70^{bA}	15.07	0.629	0.66
Tithonia diversifolia	60.0±8.17^{aB}	65.6±3.59^{abAB}	68.3±3.42^{abAB}	76.3±4.30^{bA}	73.3±2.49^{bAB}	14.51	0.186	1.78
Master®*	65.0±9.57^{aA}	61.0±5.74^{aA}	48.0±2.88^{cAB}	63.3±5.26^{cA}	42.3±5.0^{cB}	18.37	0.067	2.76
Control (Water)	70.0±5.77^{aB}	72.3±1.99^{aB}	77.0±3.86^{aB}	96.0±1.63^{aA}	100.0±0.0^{aA}	9.99	< 0.001	17.69
LSD	25.63	12.14	10.86	12.28	14.00	-	-	-
P-value	0.867	0.257	0.001	0.001	< 0.001	-	-	-
F-value	0.44	1.38	5.48	5.30	11.06	-	-	-

Means accompanied by similar letter(s) in each row (uppercase) and column (lowercase) are not significantly different. Fisher's protected Least Significant Difference test at $P \leq 0.05$. Master®* = Synthetic pesticide.

Table 4.15 Mean disease severity for early blight assessed on Tigoni potato variety treated with plant extracts and antagonistic fungi between May and August 2018 (season one)

Treatment	Weeks after planting (Mean±S.E)					LSD	P-value	F-value
	8	9	10	11	12			
Trichoderma hamatum	40.0±0.0^{aA}	44.0±1.81^{abA}	42.0±6.83^{aA}	52.0±5.08^{bA}	54.0±6.07^{cA}	14.30	0.197	1.72
Trichoderma afroharzianum	45.0±5.0^{aA}	45.7±3.42^{aA}	45.3±6.35^{aA}	46.0±2.07^{bA}	54.3±2.63^{cA}	12.66	0.492	0.89
Trichoderma asperellum	45.0±5.0^{aA}	46.7±3.81^{aA}	49.3±10.51^{aA}	48.0±3.57^{bA}	55.0±4.16^{cA}	18.09	0.802	0.41
Azadirachta indica	45.0±5.0^{aB}	40.0±1.44^{abcB}	39.0±5.0^{abB}	41.7±3.10^{bB}	59.3±4.30^{bcA}	12.07	0.016	4.33
Pistacia lentiscus	40.0±0.0^{aB}	37.0±1.26^{bcB}	38.3±4.12^{abB}	41.7±3.15^{bB}	59.7±3.54^{bcA}	8.64	0.001	10.53
Tithonia diversifolia	40.0±0.0^{aB}	45.3±4.25^{aB}	50.3±5.18^{aB}	51.3±6.543^{bB}	71.0±7.33^{abA}	16.03	0.010	4.88
Master®*	40.0±0.0^{aA}	35.33±1.59^{cA}	24±2.24^{bB}	21.7±1.37^{cB}	19.3±1.93^{dB}	4.89	< 0.001	31.28
Control (Water)	40.0±0.0^{aD}	45.0±2.40^{aDC}	52.0±4.46^{aC}	66.3±2.33^{aB}	78.0±1.93^{aA}	7.95	< 0.001	35.38
LSD	8.94	7.96	17.61	10.94	12.78	-	-	-
P-value	0.661	0.042	0.063	< 0.001	< 0.001	-	-	-
F-value	0.71	2.53	2.27	11.30	15.54	-	-	-

Means accompanied by similar letter(s) in each row (uppercase) and column (lowercase) are not significantly different. Fisher's protected Least Significant Difference test P ≤ 0.05. Master®* = Synthetic pesticide.

Table 4.16 Mean disease severity for late blight assessed on Shangi potato variety treated with plant extracts and antagonistic fungi between April and July 2019 (Season three)

Treatment	Weeks after planting (Mean±S.E)					LSD	P-value	F-value
	8	9	10	11	12			
Trichoderma hamatum	19.3±0.74^{bD}	58.5±1.96^{abC}	82.2±2.57^{bB}	86.7±1.28^{bAB}	87.4±0.74^{bA}	5.11	< 0.001	321.40
Trichoderma afroharzianum	21.5±1.48^{bC}	54.8±4.12^{abB}	78.5±6.33^{bA}	83.0±4.12^{bA}	85.9±2.67^{bA}	12.87	< 0.001	44.07
Trichoderma asperellum	20.0±0.0^{bD}	51.9±2.67^{bC}	74.8±2.67^{bB}	80.7±2.96^{bAB}	83.0±1.96^{bA}	7.31	< 0.001	131.19
Azadirachta indica	20.7±0.75^{bC}	59.3±2.67^{abB}	76.3±6.46^{bA}	81.5±5.19^{bA}	84.4±3.85^{bA}	13.45	< 0.001	37.98
Pistacia lentiscus	26.7±2.22^{aC}	62.2±3.85^{abB}	77.8±3.35^{bA}	83.0±3.92^{bA}	85.2±2.67^{bA}	10.33	< 0.001	54.66
Tithonia diversifolia	21.5±0.74^{bC}	55.6±3.40^{abB}	81.5±1.48^{bA}	85.9±1.96^{bA}	87.4±1.48^{bA}	6.35	< 0.001	192.12
Master®*	22.2±2.22^{bA}	30.4±5.34^{cA}	27.4±3.23^{cA}	25.9±1.96^{cA}	24.4±1.28^{cA}	9.90	0.472	0.96
Control (Water)	23.0±1.96^{abC}	63.7±4.86^{aB}	94.8±0.74^{aA}	100±0.0^{aA}	100±0.0^{aA}	7.46	< 0.001	199.66
LSD	4.44	11.30	11.62	9.32	6.48	-	-	-
P-value	0.074	< 0.001	< 0.001	< 0.001	< 0.001	-	-	-
F-value	2.36	7.78	26.33	50.25	112.91	-	-	-

Means accompanied by similar letter(s) in each row (uppercase) and column (lowercase) are not significantly different. Fisher's protected Least Significant Difference test at P ≤ 0.05. Master®* = Synthetic pesticide.

Table 4.17 Mean disease severity for early blight assessed on Tigoni potato variety treated with plant extracts and antagonistic fungi between April and July 2019 (Season three)

Treatment	Weeks after planting (Mean±S.E)					LSD	P-value	F-value
	8	9	10	11	12			
Trichoderma hamatum	14.8±1.96^{aD}	44.4±3.40^{bBC}	40.0±5.13^{bC}	56.3±6.46^{bAB}	65.2±4.51^{bA}	14.35	< 0.001	17.69
Trichoderma afroharzianum	25.9±3.23^{aC}	50.4±3.23^{abB}	49.6±7.30^{bB}	60.0±5.88^{bAB}	70.4±3.23^{bA}	15.38	0.001	11.41
Trichoderma asperellum	27.4±8.54^{aC}	50.4±4.86^{abAB}	41.5±5.19^{bBC}	57.8±5.88^{bAB}	65.9±3.92^{bA}	18.56	0.008	6.40
Azadirachta indica	28.2±1.96^{aC}	54.1±5.93^{abAB}	45.2±7.73^{bBC}	59.3±7.73^{bAB}	66.7±5.88^{bA}	19.58	0.012	5.69
Pistacia lentiscus	21.5±4.86^{aC}	43.0±5.34^{bB}	46.7±6.42^{bB}	61.5±7.07^{bAB}	69.6±6.06^{bA}	18.91	0.002	9.58
Tithonia diversifolia	24.4±7.14^{aC}	46.7±5.88^{abB}	50.4±2.96^{bB}	65.9±3.23^{bA}	73.3±3.40^{bA}	15.20	< 0.001	15.47
Master®*	17.0±1.96^{aAB}	21.5±2.67^{cA}	20.0±0.0^{cAB}	19.3±2.67^{cAB}	14.8±1.96^{cB}	6.60	0.258	1.56
Control (Water)	21.5±8.15^{aC}	57.8±2.22^{aB}	71.1±5.59^{aB}	90.4±1.48^{aA}	93.3±1.28^{aA}	14.54	< 0.001	40.08
LSD	16.28	13.23	16.67	16.43	12.29	-	-	-
P-value	0.608	0.001	0.001	< 0.001	< 0.001	-	-	-
F-value	0.97	6.27	6.44	12.49	29.28	-	-	-

Means accompanied by similar letter(s) in each row (uppercase) and column (lowercase) are not significantly different. Fisher's protected Least Significant Difference test at P ≤ 0.05. Master®* = Synthetic pesticide.

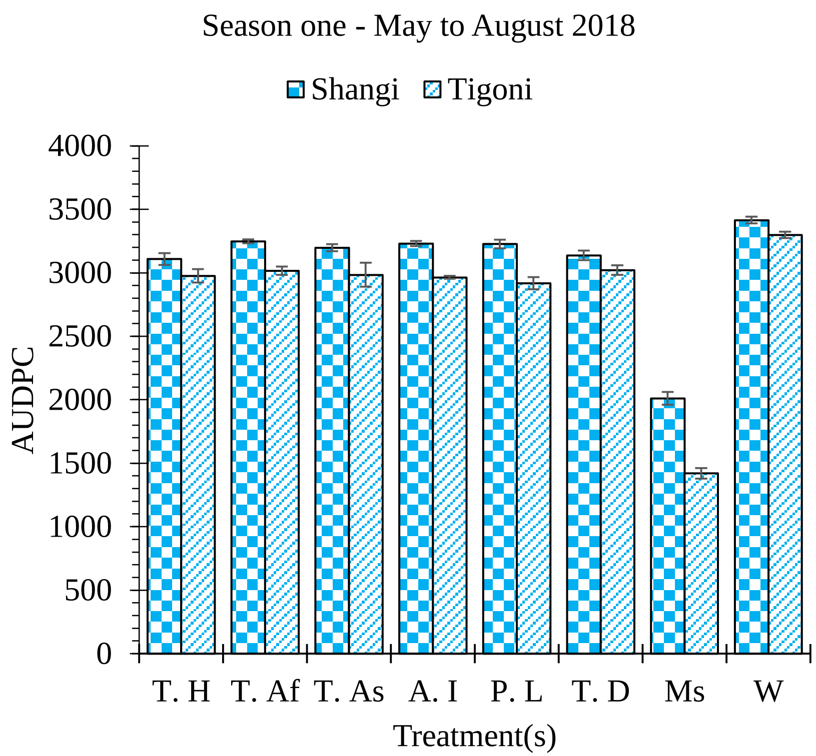
4.5.3 Area under the disease progress curve (AUDPC)

Treatments showing high area under disease progress curve (AUDPC) reflects more disease occurred hence less effective and vice versa. All the treatments of antagonistic fungi and plant extracts resulted to significant (F=164.18; df=7, 23: 31; P=0.001) reduction AUDPC when compared to the negative control (water) in both May to August 2018 and April to July 2019 (Figure 4.7). Generally, higher AUDPCs were recorded on Shangi variety than on Tigoni variety in both seasons. On both varieties, Tata Master® (positive check synthetic fungicide) resulted to the lowest AUDPC. There was a significant difference (F=164.18; df=7, 23: 31; P=0.0001) in the AUDPCs from the fungal antagonists and plant extracts compared to the positive and the negative controls (Figure 4.7).

During May to August 2018, plant extracts resulted to higher AUDPC than the antagonistic fungi on Shangi variety while on Tigoni variety, antagonistic fungi caused higher AUDPC than the plant extracts. *Trichoderma afroharzianum* caused higher AUDPCs among the fungal isolates having 3248.52 on Shangi and 3016.42 on Tigoni varieties. *Azadirachta indica* having 3231.15 and *T. diversifolia* having 3021.86 caused highest AUDPCs on Shangi and Tigoni varieties, respectively, compared to other plant extracts (Figure 4.7).

During April to July 2019, plant extracts recorded higher AUDPC than fungal isolates on both Shangi and Tigoni variety. On Shangi variety, *T. hamatum* and *P. lentiscus* having 2864.91 and 2840.83 caused the highest AUDPCs among the fungal isolates and plant extracts respectively. On Tigoni variety, *T. afroharzianum* having 2695.45 and *T. diversifolia* with 2759.76 caused the highest AUDPCs among both the fungal isolates and plant extracts, respectively (Figure 4.7).

The controls, both positive check (Tata Master®) and the negative (water) resulted to lowest and highest AUDPCs, respectively, on both Shangi and Tigoni varieties during May to August 2018 and April to July 2019. During May to August 2018, Master® caused an AUDPC of 2010.12 on Shangi and 1420.97 on Tigoni whereas water had an AUDPC of 3415.08 (Shangi) and 3415.08 (Tigoni), respectively. During April to July 2019, Master® had an AUDPC of 1966.89 on Shangi and 1995.66 on Tigoni whereas water had an AUDPC of 3042.39 on Shangi and 2888.79 on Tigoni respectively (Figure 4.7).



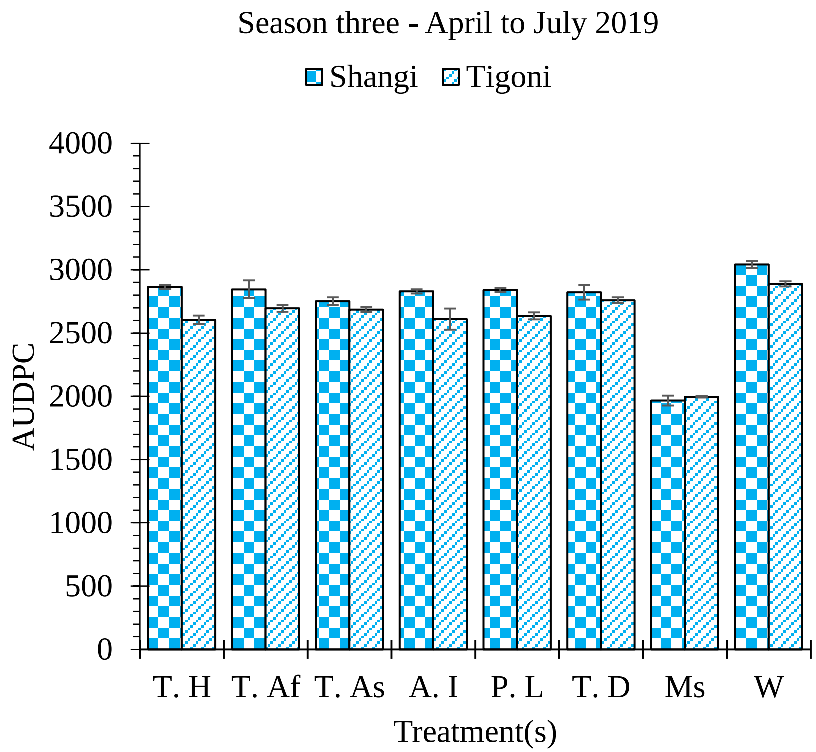


Figure 4.7 Area Under the Disease Progress Curves (AUDPC) on Shangi and Tigoni potato varieties treated with plant extracts and antagonistic fungi during May to August 2018 and April to July 2019

***T. H** = *T. hamatum*, **T. Af** = *T. afroharzianum*, **T. As** = *T. asperellum*, **P. L** = *P. lentiscus*, **T. D** = *T. diversifolia*, **Ms** = Master® and **W** = Water. Error bars denote standard errors.

4.5.4 Yield of Shangi and Tigoni potato varieties

Generally, Shangi variety had higher overall yield than Tigoni in the first season between May and August 2018, while Tigoni variety yielded higher in season three between April and July 2019 (Figure 4.8). Percentage yield over control (%Y/C) with water as the control, was higher on Tigoni variety during May to August 2018 and on Shangi variety during season two (Figures 4.11). Mistress® resulted to the highest overall total yield in both varieties in both seasons. There was a significant (F=242.11; df=7, 23; P=0.001) total yield difference between the fungal isolates and plant extracts when compared to the controls (Mistress and Water) of both potato varieties in both seasons (Figure 4.8).

Plant extracts caused higher total yield on Shangi variety whereas fungal isolates yielded higher on Tigoni variety in both May to August 2018 and April to July 2019 (Fig 4.10). *Trichoderma harzianum* having 3.31±1.06t/ha on Tigoni and 3.65±1.5t/ha on Shangi caused the highest overall yield in May to August 2018 while in April to July 2019 *T. asperellum* with 1.43±0.09t/ha on Shangi and *T. diversifolia* having 5.75±1.29t/ha on Tigoni produced the highest overall total yields compared to other treatments (Figure 4.8). Percentage yield over control (%Y/C) was higher on Tigoni variety in May to August 2018 and on Shangi variety in April to July 2019 (Figure 4.9). In May to August 2018, highest %Y/C was caused by *T. hamatum* having 97% on Tigoni variety and *T. hamatum*/*T. diversifolia* having 53% on Shangi while in season two, highest %Y/C was caused by *T. asperellum* with 219% on Shangi and *T. diversifolia* having 192% on Tigoni compared to the treatments (Figure 4.9).

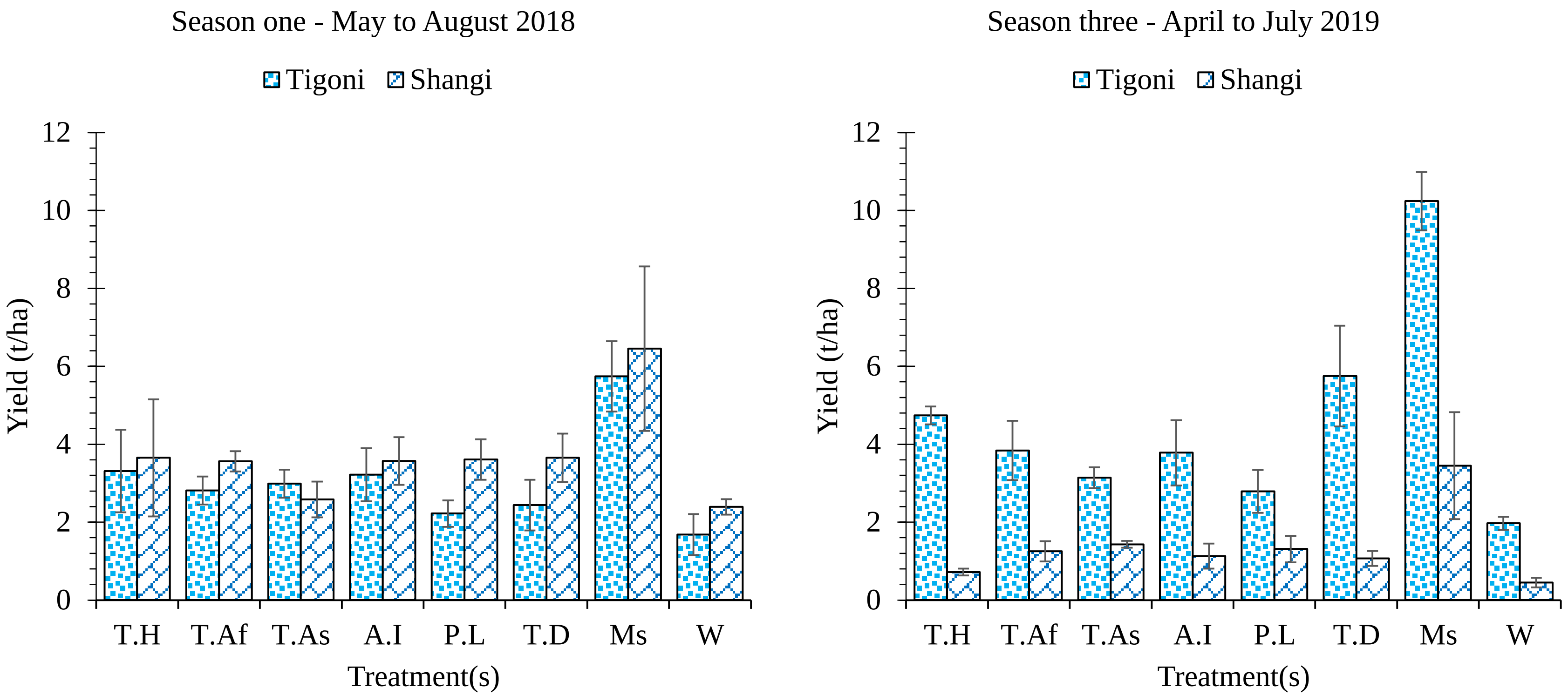


Figure 4.8 Mean total yield (t/ha) on Shangi and Tigoni potato varieties treated with plant extracts and antagonistic fungi during May to August 2018 and April to July 2019

***T.H** = *T. hamatum*, **T.Af** = *T. afroharzianum*, **T.As** = *T. asperellum*, **P.L** = *P. lentiscus*, **T.D** = *T. diversifolia*, **Ms** = Master® and **W** = Water. Error bars denote standard errors.

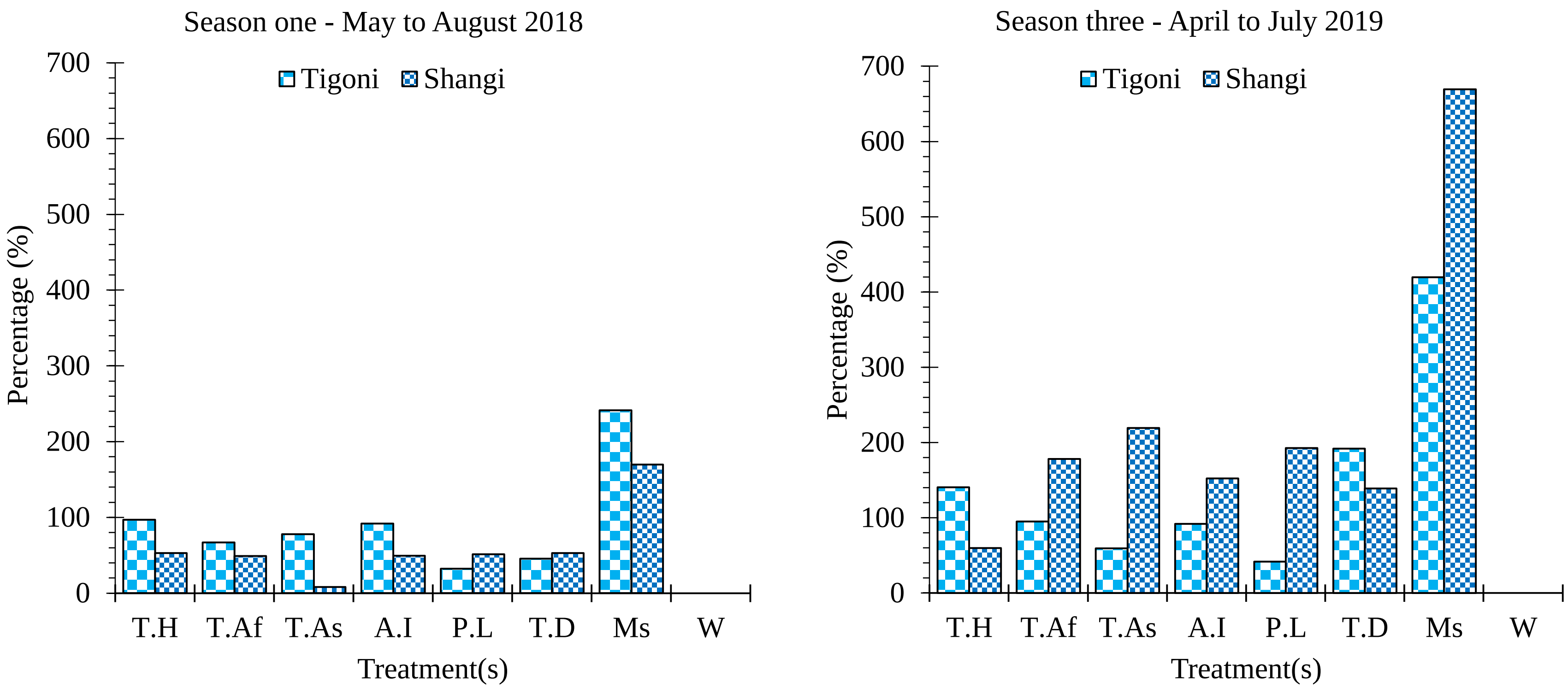


Figure 4.9 Mean percentage yield over control (%Y/C) on Shangi and Tigoni potato varieties treated with plant extracts and antagonistic fungi during May to August 2018 and April to July 2019

***T.H** = *T. hamatum*, **T.Af** = *T. afroharzianum*, **T.As** = *T. asperellum*, **P.L** = *P. lentiscus*, **T.D** = *T. diversifolia*, **Ms** = Master® and **W** = Water (Control).

On Shangi potato variety, all the treatments except *Trichoderma afroharzianum* produced higher marketable yield than on Tigoni variety in season one (between May and August 2018). However, during the third season (April to July 2019), contrary to the first season, higher marketable yield was recorded on Tigoni variety than on Shangi variety across all treatments (Figure 4.10). There was a significant (F=267.39; df=7, 23; P=0.001) yield difference in the marketable yield of both potato varieties during both May to August 2018 and April to July 2019.

Plant extracts lead to the production more marketable yields that fungal isolates in both May to August 2018 and April to July 2019. However, Master® caused the highest marketable yield while water resulted in the least marketable yields on both varieties during both seasons. During May to August 2018, *T. hamatum* produced higher marketable yield having 2.65±0.85t/ha on Tigoni and 2.99±1.36t/ha on Shangi compared to the other fungal isolates while during April to July 2019, *T. hamatum* with 4.26±0.13t/ha on Tigoni and *T. asperellum* having 0.82±0.06t/ha on Shangi yielded highest. *Azadirachta indica* resulting to 2.54±0.75t/ha on Tigoni and 2.93±0.57t/ha on Shangi variety produced higher marketable yield in May to August 2018 while *T. diversifolia* resulting to 4.54±0.33t/ha on Tigoni and *P. lentiscus* having 0.74±0.03t/ha on Shangi produced higher marketable yield in April to July 2019 as compared to the other plant extracts on both varieties (Figure 4.10).

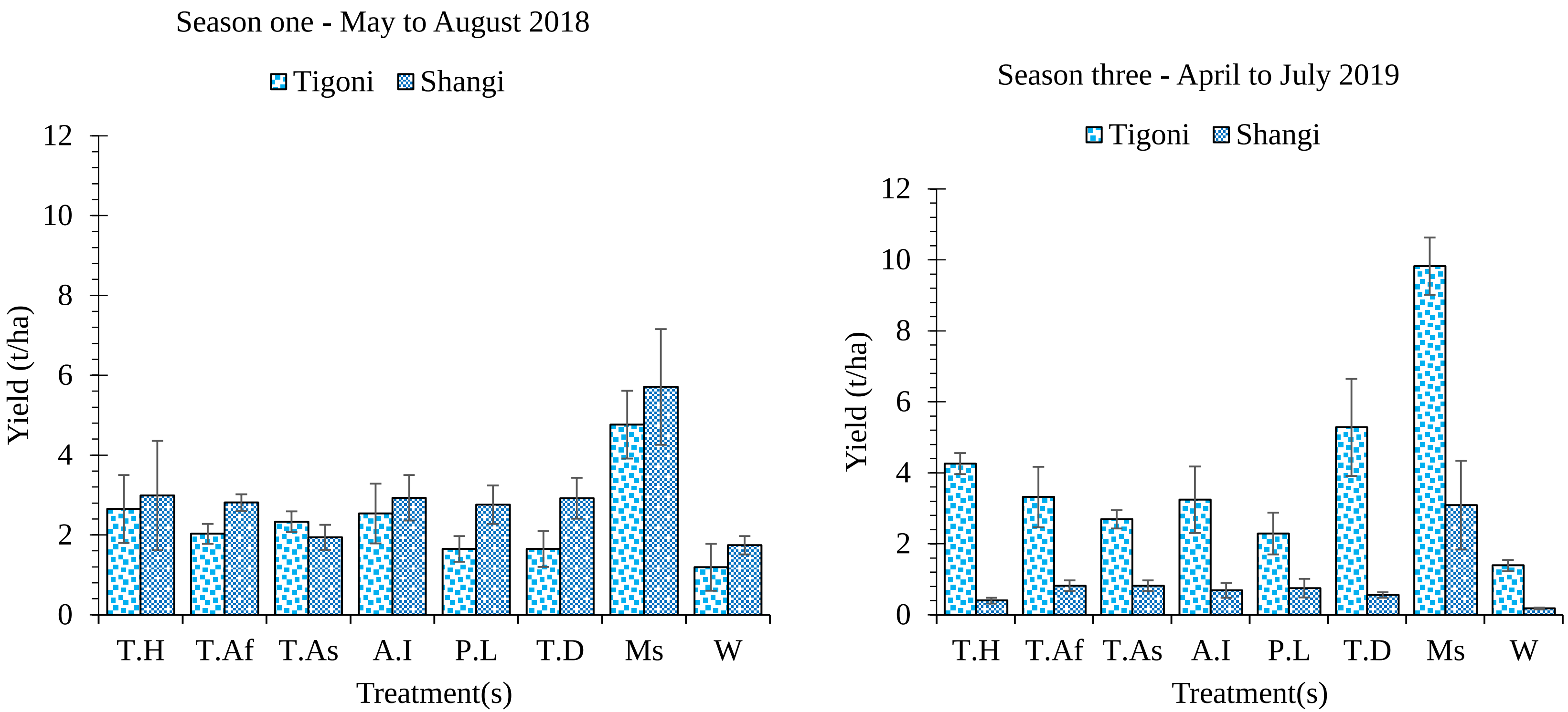


Figure 4.10 Mean total marketable yield (t/ha) on Shangi and Tigoni potato varieties treated with plant extracts and antagonistic fungi during May to August 2018 and April to July 2019

***T.H** = *T. hamatum*, **T.Af** = *T. afroharzianum*, **T.As** = *T. asperellum*, **P.L** = *P. lentiscus*, **T.D** = *T. diversifolia*, **Ms** = Master® and **W** = Water. Error bars denote standard errors.

Generally, in May to August 2018, Shangi potato variety produced higher unmarketable yield than Tigoni variety while during April to July 2019 Tigoni had higher unmarketable yield (Figure 4.11). Treatments of plant extracts resulted higher unmarketable yield compared to those of fungal isolates in both May to August 2018 and April to July 2019. During May to August 2018, *T. diversifolia* yielded 0.86±0.17t/ha on Shangi and *T. afroharzianum* recorded 0.78±0.13 t/ha on Tigoni whereas in April to July 2019, *T. afroharzianum* with 0.52±0.02t/ha on Tigoni and *T. asperellum* having 0.69±0.04t/ha on Shangi recorded the highest unmarketable yield among the treatments apart from Master® (the standard check) (Figure 4.11).

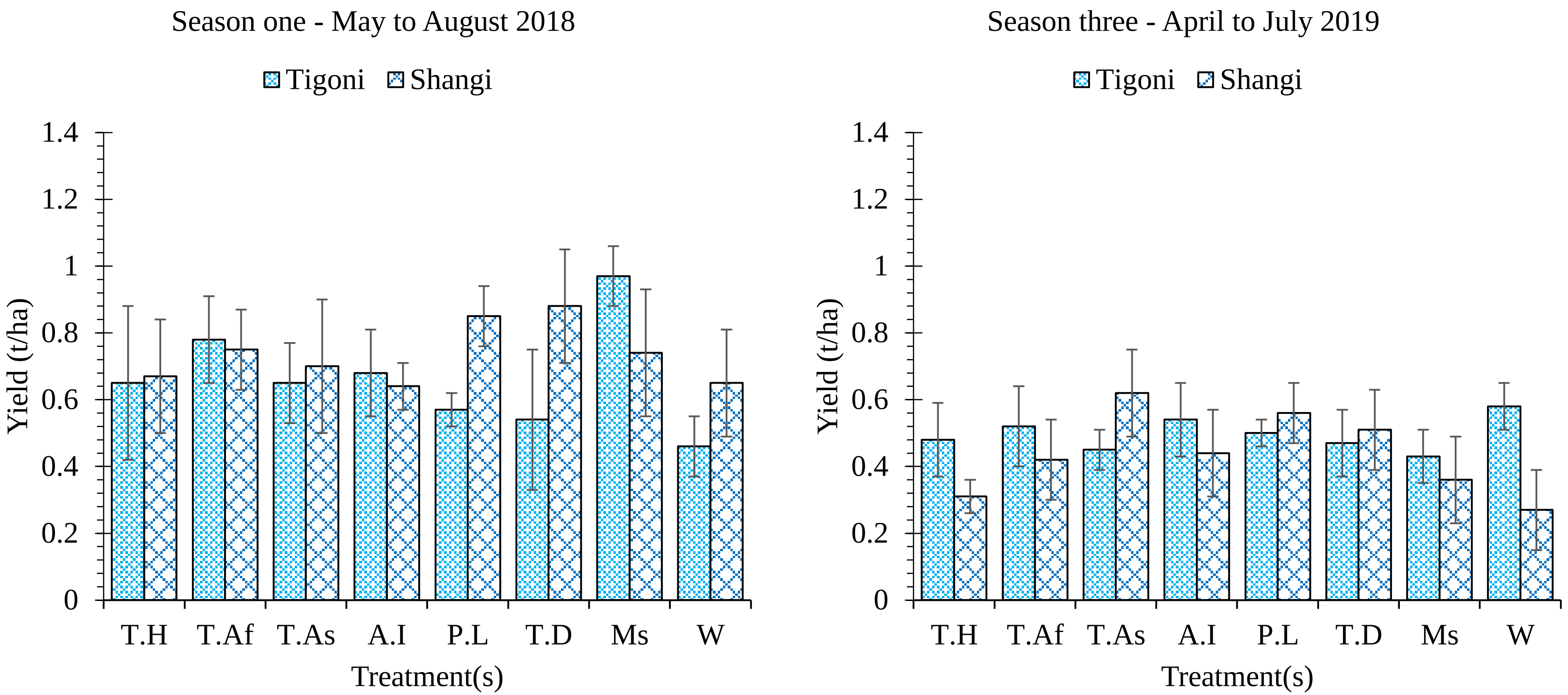


Figure 4.11 Mean total unmarketable yield (t/ha) on Shangi and Tigoni potato varieties treated with plant extracts and antagonistic fungi during May to August 2018 and April to July 2019

***T.H** = *T. hamatum*, **T.Af** = *T. afroharzianum*, **T.As** = *T. asperellum*, **P.L** = *P. lentiscus*, **T.D** = *T. diversifolia*, **Ms** = Master® and **W** = Water. Error bars denote standard errors.

CHAPTER FIVE: DISCUSSION

5.1 Factors influencing the prevalence of potato late blight Nyandarua County

The prevalence and impact of potato late blight is predetermined by various diverse dynamics. Late blight management practices and plant debris management among others also influence the incidence and severity of potato late blight in Nyandarua County.

Level of education influences access, synthesis and adoption of new technologies and information regarding agricultural productivity (Mburu *et al.,* 2014; Minai *et al.,* 2014). Current findings on levels of education indicating that a majority of the respondents attained primary level training were similar to the findings of Nyamwamu *et al.* (2014) and Okpachu *et al.* (2014) in Kenya and in Nigeria, respectively. The level of education influences decision making which plays a critical role in rural development (Taiy *et al.*, 2017). These factors can influence the incidence and severity of potato late blight eventually impacting the small-scale potato farmers.

Small-scale farmers in Nyandarua County majorly rely on potato production for food and income due to potato's short maturity period compared to other major crops such as maize, wheat and rice in Kenya (Tesfaye *et al.,* 2010; Biniam *et al.,* 2014; Taiy *et al.*, 2017). Additionally, Muthoni *et al.* (2013) reported that potato generates more income compared to other major crops in Kenya. The above findings are in agreement with those from the current study since 47.6% and 43.8% of farmers allocated between <1 acres and 1-3 acres, respectively (Omiti *et al.,* 2009; MoALF, 2016; Okello *et al.*, 2016).

Mono cropping of potato in Nyandarua County is essentially characterised by the small parcels of land owned by farmers which cannot enable other cropping practices such as crop rotation. Intensive mono-cropping of potato, however, sustains inoculum build-up and spread of late blight (Xie *et al.*, 2017). *P. infestans* can survive in living host tissue, such as seed tubers, cull piles, volunteer potatoes that are left in the field off season, on alternate hosts crops and around the root zone in the soil (Kirk *et al*., 2013; Binyam, 2014; Johnson, 2020). Therefore, mono cropping supports continuity of the pathogen between crops across different seasons.

Potato cultivation in Nyandarua County largely takes place year-round (three seasons annually), this agrees with the findings of Wakahiu *et al.* (2007). Intensive mono-cropping of potato sustains inoculum build-up of late blight (Behera and France, 2016; Xie *et al.*, 2017). Potato late blight is a polycyclic disease (the ability to produce more than one infection cycle per crop cycle), with the ability to survive in the soil and the phyllosphere by means of oospores and on alternate hosts, respectively. Hence, production of potatoes all year allows for disease recurrence and extends exposure time of late blight (Pacilly *et al.*, 2016) making it difficult to manage.

Crop rotation and fallowing are as well practiced by a good number of small-scale farmers agreeing with reports of Wakahiu *et al.* (2007) and Mwangi *et al.* (2008) in Kenya which influences the incidence of potato late blight. The practice of crop rotation with non-host crops outside the Solanaceae family such as maize, peas, peanuts and beans influence potato late blight by disrupting the disease cycle, besides improving soil fertility (Mwaniki *et al.*, 2017; Xie *et al.*, 2017).

Most farmers depend on rain fed agriculture in Nyandarua County (MoALF, 2016) although a few farmers practise irrigation during the dry seasons specifically by employing overhead irrigation. Irrigation could create a microclimate favouring the establishment and development late blight disease. Overhead irrigation can wash sporangia down to the soil which subsequently could infect potato tubers. Hence, farmers are advised to avoid/minimize overhead irrigation but instead consider surface irrigation which is less costly and limits the spread of the pathogen's inocula.

Shangi is the most preferred potato variety by the small-scale potato farmers majorly because it is high yielding, it matures early and it has a ready market (Janssens *et al.*, 2013; Musita *et al.*, 2019; Mutegi *et al.*, 2021). Higher prices offered for Shangi variety was reported as another major reason for its preference (MoALF, 2016). Despite its preference, Shangi is classified as a moderately susceptible variety against potato late blight by the National Potato Council of Kenya (NPCK, 2017). However, according to the current study, Shangi portrayed a highly susceptible variety to late blight, hence, with the notable aggressive nature of pathogen under favourable conditions, can be attacked and devastated by the late blight disease.

The findings from this study revealed that potato seeds are mainly sourced from famers' previous harvests, this in agreement with the findings of Muthoni *et al.* (2013) while assessing potato production in Kenya. Few farmers' purchase seeds for their subsequent planting seasons from certified dealers, this is similar to the findings of Kinyua *et al.* (2008), Chindi *et al.* (2013), Schulte-Geldermann *et al*. (2012) and Nyamwamu *et al.* (2014). The use of recycled seeds is preferred by most small-scale farmers because of the cost implications in purchasing certified seeds (Mburu *et al.,* 2014).

Recycling of seed is one of the major reasons for low yield in potato production, since most seed-borne pathogens, including potato late blight are most likely to survive on seeds off season and could be carried on to the following planting season (Gaur, 2010; Schulte-Geldermann *et al*., 2012; Nyamwamu *et al.,* 2014; Okello *et al.*, 2016; Thomas-Sharma *et al.*, 2016). When infected tubers which are considered to be the main means of overwintering and primary inoculum sources, are exposed to favourable conditions, the transmission rate of *P. infestans* to the subsequent seasons is up to 25%. Seed to shoot transmission can occur within 24hrs on a rainy season (Powelson *et al.,* 2002; Johnson, 2010; Johnson *et al.,* 2012).

Poor quality of seed or infected seed can cost farmers up to 50% of the total production costs (Gaur, 2010), which in turn leads to low yields hence reduced food, this escalates food insecurity (Al-Sadi, 2017). The use of certified seeds lowers the chances of occurrence of primary inoculum of *P. infestans* (Thomas-Sharma *et al.,* 2016). Infected seed tubers and plant debris are high potential sources of primary inoculum, this is supported by the findings of Cooke *et al.* (2011) who reported seed driven epidemics due to potato late blight in Europe.

Potato late blight, bacterial wilt, early blight and viruses are the major diseases causing havoc to small scale potato farmers in Kinangop Sub-County (MoALF, 2016). Findings from the current study agree with those reported by Kaguongo *et al.* (2008; 2010) and Muthoni *et al.* (2013). Due to the significant losses caused by these diseases, mainly potato late blight, farmers have resorted to excessive application of fungicides (Kaguongo *et al.,* 2008; Muthoni *et al.,* 2013). Majority of the farmers apply fungicides on a seven-day regime (weekly) during the rainy season whereas during the dry season it's applied on a twice a month regime. These findings are in agreement with those of Namanda *et al.* (2004) who reported that in Uganda, pesticides were being applied more than ten times in a single growing season.

Overuse of fungicides from a single group has been associated with resistance development by *P. infestans* and new and more aggressive biotypes have emerged in the recent years in Ethiopia (Mekonen and Tedesse, 2018). One of the chemical late blights has been reported to be resistant to in Kenya is Metalaxyl, reported on isolates of the US-1 lineage (Njoroge, 2019). Overuse of fungicides, way above the recommended rates on the labels, is not economically viable for small scale farmers due to the cost implications increasing the cost of production. Additionally, synthetic fungicides are harmful to human health causing long term chronic illnesses and since synthetics are not biodegradable and lack target specificity, they lead to environmental pollution affecting biodiversity.

Therefore, farmer practices such as knowledge level and size of land allocated to potato production, production and disease management practices such as mono-cropping, crop rotation, irrigation, recycling of farm saved seeds for planting, variety choice, fungicide application and application regime have an impact on the prevalence and impact of potato late blight. According to the current findings, late blight *P. infestans* is the major potato disease causing havoc to famers in Nyandarua County which is mainly managed by synthetic fungicides, hence the need for other safer integrated management options.

5.2 Physicochemical characteristics of the soil at the experimental site in KALRO-Tigoni Horticultural center

Since the field experiments were conducted under natural infestation, abiotic factors such as soil pH, soil texture and nutrients could possibly have had some influence on persistence of *P. infestans* in the soil and root rhizosphere (Andrivon, 1995; Oyarzún *et al.,* 2011). Soil texture supports the persistence of *P. infestans* zoospore and sporangia. Therefore, the sandy clay soil type from study site (Table 4.9), having a good water-holding capacity and porosity could have supported the persistence of *P. infestans* over a long period of time hence the presence of the pathogen under favourable environmental conditions. High acidity (low pH) is toxic to plants growth and development and may affect nutrient availability and toxicity in the soil (Matsumoto *et al.,* 2017; Gentili *et al.,* 2018). The low pH and calcium deficiency of the soils at the study site (Table 4.9) could have influenced *P. infestans* activity by directly inhibiting its mycelial growth on potato tubers in the soil and around the potato rhizosphere.

5.3 Activity of biological agents against *P. infestans in vitro* and under field conditions

In the current *in vitro* study, plant extracts and fungal isolates showed variation in their mycelial suppressive and inhibitory activities. The current study confirmed that *Trichoderma* genus has a rapid *in vitro* growth and sporulation rate compared to other genera; this gives it a competitive advantage over plant pathogenic fungi (Svetlana *et al.,* 2010; Hasan, 2015; Abdel-lateif, 2017). Synthetic pesticides have put a lot of pressure on human health and the environment leading to the demand for safer alternatives. To develop effective and acceptable products for crop protection, *in vitro* efficacy evaluations on the potentials of any microbes and plant extracts are necessary.

Fungi in the genus *Trichoderma* have been reported to deploy several mechanisms of action against plant diseases. These mechanisms include, mycoparasitism, cell wall degradation, antibiotic production, mycelial growth and sporulation inhibition, and indirectly by, competition, plant growth promotion, induction of plant defensive mechanisms and antibiosis (Howell, 2003; Benitez *et al.*, 2004; Vinale *et al*., 2008; Hashem *et al.*, 2016; Waghunde *et al.*, 2016). The *in vitro* results from the current study carried out using the dual culture technique showed a significant mycelial inhibition of *P. infestans* by *Trichoderma harzianum, T. afroharzianum* and *T. asperellum* at varying levels. Similar results to the findings from this study were reported by El-Naggar *et al.* (2016) and Bouziane *et al.* (2016) who observed a significant mycelial inhibition on *P. infestans* by *T. harzianum* and *T. viride in vitro*.

plant extracts showed strong antioxidant capacity both in vitro and in vivo, and the extracts can be considered a good source of natural antioxidants and antimicrobials (Altemimi *et al*., 2017). Amongst the plant extracts, *Azadirachta indica, Pistacia lentiscus* and *Tithonia diversifolia* were superior in suppressing the mycelial growth of *P. infestans in vitro. Azadirachta indica* contains azadirachtin, nimbin and salanin with azadirachtin being its main active ingredient. Neem extracts contain complex mixture of molecules, including normal hydrocarbons, phenolic compounds, terpenoids, alkaloids, and glycosides, flavonoids, and saponins (Hossain *et al.*, 2013; Pandey *et al.*, 2014). Morgan (2009) and Fernandes *et al.* (2019) reported that while a lesser amount of azadirachtin is found in Neem leaves, the compound is abundant in Neem seed oil. Reports by Salazar *et al.* (2015) and Fernandes *et al.* (2019) among others, state that due to its high concentration of terpenoids, azadirachtin disrupts cell membrane, inhibits respiration inhibiting growth.

Pistacia lentiscus predominantly consists of triterpenes which have been reported to alternate cell membrane by inhibiting lipid biosynthesis, rendering the cell porous (Haraguchi *et al.,* 1999). *Tithonia diversifolia* on the other hand, essentially consists of tagitinin A, C and F. Tagitinin C is the main abundant active ingredient (over 65%) rich in polyphenols, flavonoids and tannins (Omokhua *et al.,* 2018).

Significant mycelial suppression against *P. infestans* at varying levels were also realised in *Azadirachta indica, Pistacia lentiscus* and *Tithonia diversifolia* extracted using an ethanol-acetone mixture and evaluated using the food poison technique (Ngadze, 2014). Findings from the current study were in agreement with the reports of Abayhne *et al.* (2016) that methanol and ethanol extracts from *Cymbopogon citratus* and *Datura stramonium*, suppressed mycelial growth and directly inhibited zoospore germination of *P. infestans in vitro*.

In relation to the current study, Rashid *et al.* (2004) also reported a significant inhibitory effect on the mycelial growth, spore production and spore germination of *P. infestans* by distilled water extracts of *Azadirachta indica*. Moumene *et al.* (2015) using decoction extraction method reported higher inhibitory effects than the current study on growth, sporulation and germination of *P. infestans* by *Rosmarinus officinalis*. Phytochemicals extracted by ethanol and acetone (Table 2.1) have been reported to cause membrane disruption, inhibiting respiration and ion transport processes, enzyme inhibition, enzyme inactivation, binding to proteins, and adhesion among other actions (Yanar *et al.,* 2011; Gurjar *et al.,* 2012; Rodino *et al.,* 2013).

The inhibitory and suppressive activity of the fungal isolates and plant extracts, respectively, were further evaluated under field conditions to assess for potential activity. The results from the field evaluation reveal that plant extracts and antagonistic fungi have the potential to reduce invasion of *P. infestans* and increase yield on potato under field conditions. Yao *et al.* (2016) reported that under field conditions, *Trichoderma* strains were efficient against late blight, and significantly reduced the disease severity compared to the control. They proved *Trichoderma* was able to reduce the disease severity and disease incidence of potato plants through mechanisms such as production of antifungal metabolites and growth inhibition of *P. infestans*. Goufo *et al.* (2010), Ngadze (2014), Moumene *et al.* (2015) and Anju *et al.* (2017) reported a reduction of late blight severity and incidence by crude plant extracts on potato under field conditions which they associated with activation of plant defence responses and inhibition of spore germination.

Antagonistic fungi were generally higher than plant extracts at reducing late blight's incidence and intensity under field conditions. This could possibly be attributed to the inhibitory activity of the tested fungal strains, method used for phytochemical extraction, choice of solvents, concentration of the extracts, and the present active compounds in the extracts (Wongkaew and Sinsiri, 2014). Generally, the activities of the biological agents against potato late blight were significantly lower under field conditions compared to the *in vitro* results. Efficacy of bio-agents and plant extracts evaluated in the current study varied from *in vitro* to *in vivo* conditions and the variation could possibly be attributed to the desynchronised environments between the laboratory and the field (Lal *et al.*, 2016). In spite of the antimicrobial potential of biocontrol agents, they alone are not adequate to replace synthetic fungicides.

Findings from the *in vivo* trials clearly indicate a significantly greater effectiveness of synthetic fungicide (Tata Master® 72WP) in managing potato late blight and producing higher yields when compared to the plant extracts and antagonistic fungal isolates. Majeed *et al.* (2014; 2017) reported that managing late blight using single control method at a time cannot fully neutralize the adverse effects of late blight except for an integrated and eco-friendly approach. Soytong and Ratanacherdchai (2005) similarly stated that biological control has proven safe and effective, but to manage blight, it must be integrated with other disease control methods.

Foliar pathogens such as *P. infestans* are not easily manageable through biological control as compared to the soil dwelling pathogens. The site of treatment application/target site for foliar pathogens is the phyllosphere, mainly the phylloplane. Application on the phyllosphere exposes the biological agents to adverse environmental conditions for instance, exposure to direct sunlight influencing their colonisation rate, survival and activity. The low levels of activities showed by the microorganisms and plant extracts compared to the synthetic fungicide under field conditions could have been influenced by weather conditions. For instance, high temperatures and radiations could deactivate the biological agent; they could also be washed down by rain. Cool-humid environmental conditions favour the development of late blight, hence high disease incidence with a short and recurrent disease cycle could significantly overwhelm the biological control agents.

There was a shortage of certified potato seeds in Kenya at the time of project initiation therefore seeds used for the first and third seasons were those from the previously planted season. Hence, poor quality of seeds planted might have lowered the crop vigour hence lowering the plant defence mechanisms making it prone to disease attack. The seeds could also could have been a source of pathogen, hence aggravating the infection.

Other potential factors that could have led to the relatively low activity not resulting to complete effectiveness of the microorganisms and plant extracts compared to the synthetic fungicide could be the carrier material used, type of formulation which could have possibly dictated the stability and activity, the mode of action employed, source and method of isolation or extraction of the biological agents and the type of solvent used in the extraction process (Pal and McSpadden, 2006; Slavica and Brankica, 2013). There exist several methods of phytochemical extractions, including liquid-solid extraction and solvents extraction at high or low temperatures. However, alcohol-based solvents are preferred to water for phytochemical extraction since they are associated with higher extraction yield (Fernandes *et al.*, 2018).

Supposedly, biocontrol organisms face difficulties establishing on foliar surfaces due to several abiotic factors such as temperature fluctuations, nutrients availability, water availability and UV radiation (Köhl, 2009). In order to enhance their efficacy under field conditions, incorporation of biological control agents (BCAs) in the IDM programs would be the best option. Proper formulation and delivery at an earlier stage under favourable environmental conditions could be effective in enhancing the activity of the BCAs.

The results from this study have proven the existence of potentially bioactive microorganisms and plants within the local environment. Therefore, use of these naturally available products will guarantee clean, quality and healthy food produce with no chemical residues, hence attracting markets. Environmental pollution will also be lessened increasing productivity therefore ensuring food and nutrition security.

CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

i. Socio-demographic factors and farmers practices in crop production and disease management influence the occurrence of potato late blight in Nyandarua County.

ii. Farmers over-rely on synthetic chemical from a single group of pesticides to control late blight on potato.

iii. There exist a diverse community of fungi within the potato rhizosphere and bioactive compounds from plant materials in the local environment with antagonistic potential against *Phytophthora infestans*. *Trichoderma harzianum, T. afroharzianum*, *T. asperellum*, *A. indica, P. lentiscus,* and *T. diversifolia* showed highest potential for managing potato late blight both *in vitro* and under field conditions thus increasing yields.

iv. Fungal antagonists were more effective in the management of potato late blight compared to crude plant extracts.

6.2 Recommendations

i. Farmers should be sensitized to reduce over-reliance on synthetic pesticides and instead employ integrated disease management options, as well as good agricultural practices (GAPs) in potato production.

ii. The fungi *Trichoderma harzianum, T. afroharzianum, T. asperellum* are more effective and should therefore be prioritized in developing and commercialising late blight control products for potato farmers.

iii. Further research should be conducted using crude extracts of *Azadirachta indica, Pistacia lentiscus* and *Tithonia diversifolia* to substantiate their action against potato late blight under field conditions as they were less effective in the current work.

iv. Further research to be carried out to determine the modes of action of the selected fungi and crude plant extracts, and the potential for synergy, against potato late blight under field conditions.

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APPENDICES

Appendix I: Survey questionnaire

I am interested in your opinion about the effects of the current socio demographic factors, production and management practices of potato on the occurrence of late blight in Nyandarua County. Please take a few minutes to respond to the questions below.

Questionnaire number: …………………… Date: …………………

Section A: Details of the respondent's area

County: ……………………. Sub County: …………………………

Location: …………………… Sub-location: ………………………..

Village: ……………………… Nearest town: ……………………….

Agro ecological zone: ………………………….

GPS Coordinates: Latitude………… Longitude………….. Altitude………

Section B: Socio demographic characteristics of potato farmers

Name of the farmer: ………………………………. Contact: ……………

i. Sex: (1) Male (2) Female

ii. Age (Yrs.): (1) Below 20 (2) 20-25 (3) 26-30 (4) 31-35 (5) 36-40 (6) 41-45 (7) 46-50 (8) 51-55 (9) 56-60 (10) Over 61

iii. Education level: (1) None (2) Primary (3) Secondary (4) Post-secondary

iv. Farm ownership(s): (1) Owner (2) Hired (3) Partnership (4) Manager

v. What is the size of your owned farm (Acres)?
(1) <1 (2) 1-3 (3) 4-6 (4) 7-9 (5) >10

vi. What is the size of your leased farm (Acres)?
(1) <1 (2) 1-3 (3) 4-6 (4) 7-9 (5) >10

vii. Area under potato (Acres)?
(1) <1 (2) 1-3 (3) 4-6 (4) 7-9 (5) >10

Section C: Potato production systems

1. How many planting seasons do you have for potato?

    (1) One (2) Two (3) More than two seasons

2. For how long have you been growing potato?

    (1) One season (2) One Year (3) Two years (3) 3- 5 years

    (4) Over 5 years

3. What is your farm's source of labor?

    (1) Hired (2) Family (3) Both

4. What potato variety(s) do you prefer? (rank in order of preference)

    ____Tigoni ____Sherekea ____Pure gold

    ____Nyayo ____Shangi ____Asante

    ____Ngure ____Kenya mpya

5. Why do you prefer the above variety(s)? (tick all that apply)

Varieties	High yield	Early maturity	Ready market	Readily available	Tolera nce	Longer dormancy
Tigoni						
Asante						
Kenya mpya						
Ngure						
Shangi						
Nyayo						
Pure gold						
Sherekea						

6. Where do you get your potato tuber seeds from?

    (1) Purchase from a certified dealer (2) Buy from neighbor

    (3) Select from previous own harvest (4) Others (Specify)……….

7. If (1) Purchased from a certified dealer from above, specify the dealer……………..

8. What type of cropping systems do you practice?

    (1) Mono-cropping (2) Inter cropping (3) Relay cropping
    (4) Crop rotation

9. If you practice crop rotation, which crops do you rotate with potato? (tick all that apply)

   (1) Tomato (2) Cucumber (3) Eggplant (4) Squash
   (5) Water melon (6) Tobacco (7) Others (Specify)……………

10. Why do you prefer to rotate with this crop? (please rank in order of preference)

   ____Improve soil structure ____Nitrogen fixing

   ____ Pest, pathogen management ____Weed management

   ____Preventing soil erosion ____increase yield

   ____Risk management Other(s)……………………….

11. How long do you practice crop rotation?

   (1) One season (2) Two seasons (3) More than two seasons

12. If you practice intercropping, which crops do you intercrop with potato? (tick all that apply)

   (1) Tomato (2) Cucumber (3) Eggplant (4) Squash
   (5) Water melon (6) Tobacco (7) Other(s) …………..…………

13. Why do you prefer to intercrop with this crop? (rank in order of preference)

   ____Prevent soil erosion ____Nitrogen fixing

   ____Pest and pathogen management ____Weed management

   ____Increased yield Other(s)……………………………….

14. Do you practice fallowing?

   (1) Yes (2) No

15. For how long, if yes?

   (1) One season (2) Two seasons (3) More than two seasons

16. Do you practice irrigation?

   (1) Yes (2) No

17. If yes, what is the source of your irrigation water?

   (1) River (2) Pond (3) Dam (4) Borehole

Section D: Pests and diseases and their management

1. Which are the major diseases you've encountered in potato farming?

   (1) Late blight (2) Early blight (3) Potato wart disease
   (4) Dry rot (5) Common scab (6) Soft rot (7) Viruses
   (8) Nematodes (9) Others (Specify)…………………..……

2. Which are the major pests you've encountered in potato farming?

(1) Aphids (2) Cut worm (3) Nematodes
(4) Potato tuber moth (5) Others(Specify)……………………………

3. At what stage of crop growth do you notice the diseases?

(1) Seedling (2) Vegetative (3) Flowering (4) Harvesting (5) All stages

4. At what stage of crop growth do you notice the pests?

(1) Seedling (2) Vegetative (3) Flowering (4) Harvesting (5) All stages

5. How long has late blight disease been a problem in your farm?

(1) <1 yr. (2) 2-3yrs (3) 4-6yrs (4) >7yrs

6. On what variety (s) is it most severe? (rank in order of priority)

____Tigoni ____Sherekea ____Pure gold

____Nyayo ____Shangi ____Asante

____Ngure ____Kenya mpya

7. At what stage of the plant does the late blight disease occur?

(1) Seedling (2) Vegetative (3) Flowering (4) Harvesting
(5) All stages

8. What control measures do you apply to manage this disease?

(1) Use of fungicides (2) Use of Resistant variety(s)
(3) Uprooting affected plants (4) Use certified seeds/potato tubers
(5) Others (Specify)………...……………………………

9. If use of fungicides, which fungicide(s) do you apply?

Specify trade name:……………………………………………….

10. How effective is the fungicide(s) applied?

(1) Very effective (2) Effective (3) Less effective
(4) Not effective

11. At what frequency is the fungicide(s) applied?

Specify ……………………………………..

12. If use of resistant variety(s), which potato variety(s) do you use?

Specify …………………………………….

13. From where do you get the knowledge on potato disease management practices?

(1) Government extension staff (2) Private extension staff
(3) Field days (4) Agrovet shops (5) Radio/TV (6) Newspapers
(7) Neighbors

14. Are you aware of biological control strategies used in managing potato diseases?

(1) Yes (2) No (skip to question 19)

15. How did you know about the biological control product (s)/strategies?

(1) Government extension staff (2) Private extension staff
(3) Field days (4) Agrovet shops (5) Radio/TV

(6) Newspapers
(7) Neighbors

16. Have you used any biological control product?

    (1) Yes (2) No

17. Which biological control product(s) have you used?

    (1) Specify……………………….……………………………………

18. How effective has the biological control product (s) you've used been?

    (1) Very effective (2) Effective (3) Less effective
    (4) Not effective

19. Are you aware of the term Integrated Pest Management?

    (1) Yes (2) No (skip to question 36)

20. Have you ever used a combination of control methods to manage late blight disease on potato? (Specify)…………………………………………..

21. How do you determine when to initiate control of late blight disease on your potato? (tick all that apply)

    (1) When I see disease symptoms (2) Changing weather conditions
    (3) At a particular crop growth stage (4) Follow extension recommendations
    (5) Chemical company agronomist/salesmen recommendations
    (6) My own experience (7) Specify Others ………………….

Section E: Post harvest handling and management

1. What is your average yield of potato in **bags per Acre** (1bags……kg)?

   (1) <5 (2) 6-10 (3) 11-15 (4) 16-20 (5)21-25 (6) >26

2. Do you store or sell your potatoes immediately after harvest?

   (1) Store (2) Sell

3. If stored after harvest, for how long does the storage last?

   (1) < 1 month (2) 1-3 months (3) 3-6 months (4) >6 months

4. Where do you store the potatoes after harvest?

   (1) In-house storage (2) pits (3) granaries
   (4) Other structures: Specify…………………………………………

5. What do you do with the plant debris after harvest? (tick all that apply)

   (1) Animal feed (2) Burry (3) Leave in the field
   (4) Others (Specify)………………………………………

6. What's the distance to the nearest market?

   (1) Less than 1Km (2) 1-2Km (3) 3-4Km (4) More than 5Km

7. What is your marketing channel?

(1) Direct marketing (2) Brokers (3) Co-operative (4) Farmer group

8. What marketing constraints are you facing?

   (1) Packaging (2) Transportation (3) Buyers (4) Price fluctuations
   (5) Competition (6) Brokers (7) Storage facilities (8) Market information

9. Are you a member of any SACCO/Growers Association/Farmers group?

   (1) Yes (2) No

10. If yes in Q9, name any that you are registered with.

    ………………………………………………………………….

11. What benefits do you get from your membership in the SACCO/Growers Association/Farmers group you're in?

    (1) Extension services (2) Inputs (3) Loans (4) Marketing (5) insurance
    (6) Others: Specify…………………………………………………….

12. Do you get any financial aid to support your potato production?

    (1) Yes (2) No

13. If yes, where do you get your financial aid from?

    (1) Self (2) friends and relatives (2) Banks (3) Sacco
    (4) Government (5) Specify others…………………..

14. Is there any other information you may want to provide regarding potato production in this area?

……

Thank you for your participation in this survey.

Appendix II: Research authorization letter from Kenyatta University

KENYATTA UNIVERSITY
GRADUATE SCHOOL

E-mail: dean-graduate@ku.ac.ke
Website: www.ku.ac.ke

P.O. Box 43844, 00100
NAIROBI, KENYA
Tel. 8710901 Ext. 57530

Our Ref: A145/37650/2016

DATE: 8th February, 2018

Director General,
National Commission for Science, Technology
and Innovation
P.O. Box 30623-00100
NAIROBI

Dear Sir/Madam,

RE: RESEARCH AUTHORIZATION FOR OCHIENG STEVE AGONG – REG. NO. A145/37650/2016

I write to introduce Mr. Ochieng Stve Agong who is a Postgraduate Student of this University. He is registered for M.Sc degree programme in the **Department of Agricultural Science and Technology.**

Mr. Ochieng intends to conduct research for a M.Sc Proposal entitled, **"Biocontrol of Late Blight *Phytophthora infestans* on Potato using Selected Fungal Antagonists and Plant Extracts in Kiambu County, Kenya".**

Any assistance given will be highly appreciated.

Yours faithfully,

MRS. LUCY N. MBAABU
FOR: DEAN, GRADUATE SCHOOL
AM/rwm

Appendix III: Research permit from National Commission for Science, Technology and Innovation (NACOSTI)

NATIONAL COMMISSION FOR SCIENCE,
TECHNOLOGY AND INNOVATION

Telephone:+254-20-2213471,
2241349,3310571,2219420
Fax:+254-20-318245,318249
Email: dg@nacosti.go.ke
Website : www.nacosti.go.ke
When replying please quote

NACOSTI, Upper Kabete
Off Waiyaki Way
P.O. Box 30623-00100
NAIROBI-KENYA

Ref: No. **NACOSTI/P/19/77730/28093**

Date: **13th March, 2019**

Steve Ochieng' Agong
Kenyatta University
P.O. Box 43844-00100
NAIROBI.

RE: RESEARCH AUTHORIZATION

Following your application for authority to carry out research on ***"Biocontrol of late blight phytophthora infestans on potato using selected fungal antagonists and plant extracts In Kiambu County, Kenya"*** I am pleased to inform you that you have been authorized to undertake research in **Kiambu and Nyandarua Counties** for the period ending **12th March, 2020.**

You are advised to report to **the County Commissioners and the County Directors of Education, Kiambu and Nyandarua Counties** before embarking on the research project.

Kindly note that, as an applicant who has been licensed under the Science, Technology and Innovation Act, 2013 to conduct research in Kenya, you shall deposit **a copy** of the final research report to the Commission within **one year** of completion. The soft copy of the same should be submitted through the Online Research Information System.

GODFREY P. KALERWA MSc., MBA, MKIM
FOR: DIRECTOR-GENERAL/CEO

Copy to:

The County Commissioner
Kiambu County.

The County Director of Education
Kiambu County.

National Commission for Science, Technology and Innovation is ISO9001: 2008 Certified