CHARACTERISATION OF *Phaeoisariopsis griseola,* PHYTOCHEMICAL SCREENING AND FUNGICIDAL ACTIVITY OF SELECTED PLANT EXTRACTS AGAINST *Phaeoisariopsis griseola* OF COMMON BEAN Var. GLP 1127 (Mwezi moja) MASENO, KENYA

BY

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DECLARATION

I, the undersigned, declare that this thesis is my original work and that it has never been presented in any University or institution for academic credit. All sources of information have been acknowledged by means of references and citation.

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DEDICATION

To my parents, wife Lynet and son Fidel Meso.

ABSTRACT

Angular leaf spot disease (ALS) caused by *Phaeoisariopsis griseola* is a major constrain of common bean leading to production of less than 25% of the potential yield thus posing a chronic threat to food security in Kenya. Synthetic fungicides are primarily preferred in the management of ALS although it's use is becoming restrictive due to a number of shortcomings. *Azadirachta indica, Allium sativum* and *Tithonia diversifolia* plant extracts have demonstrated successful antimicrobial activity against plant pathogens and are gaining popularity as they are safe to use, easily available and cheap. Little though is known about the success of using these crude extracts to manage ALS. The aim of this study was to characterise *Phaeoisariopsis griseola*, phytochemically screen and determine fungicidal activity of *Azadirachta indica, Allium sativum* and *Tithonia diversifolia* extracts against *Phaeoisariopsis griseola*. The study was conducted at Maseno University. Plant specimens were taxonomically identified and authenticated then air-dried and fine ground for extraction using methanol, ethanol and distilled water. *Phaeoisariopsis griseola* from Ugenya, Bondo and Sabatia sub-counties were isolated for morphological profiling and pathogenicity tests. The plants specimens were screened for phytochemicals. Inhibition of spore germination of *Phaeoisariopsis griseola* was determined at concentrations of 50, 75 and 100% In *vitro*. Synthetic fungicide and water were used as positive and negative controls respectively. *In vivo* evaluations were conducted in the greenhouse in a completely randomized design with four replications. The experimental plants were inoculated with 60ml suspension 2.5 x 10^6 spore/ml of *Phaeoisariopsis griseola* 5 weeks from emergence. Two weeks later, the plants were sprayed with 100% concentration methanolic extracts. Data on morphological profiling was assessed under a light microscope after 14 days incubation at 25^{o}C. Data on pathogenicity tests was scored on being pathogenic or not pathogenic. Data on phytochemical screening was scored for presence or absence of specific phytochemicals, Inhibition zones were measured in millimetres, growth, yield and disease index were collected 8 weeks from emergence and the data collected subjected to Analysis of Variance (ANOVA) and treatment means separated and compared using LSD (P=0.05). Morphological profiling of isolates revealed variations in terms of hyphae and spores' characters. Alkaloids, tannins, flavonoids, terpenoids, saponins, cardiac glycosides and sterols were present in nearly all the plant specimens with flavonoids being absent in *Azadirachta indica* and sterols, saponins and alkaloids being absent in *Tithonia diversifolia*. All the plant extracts significantly inhibited spore germination of *Phaeiosariopsis griseola* ($P \leq 0.05$). The most effective was methanolic *Allium sativum* extract at 100% concentration. Among the plant extracts *Allium sativum In vivo* had the highest fungicidal effect proportionate to the concentration percentages. Methanol had the highest extraction potential while water solvent had the least effect. The extracts had higher significant effect on growth index, yield components and disease index ($P<0.001$). From this study plant extracts of *Azadirachta indica, Tithonia diversifolia* and *Allium sativum* had higher potential for pathogen control at higher concentrations (100%) hence are recommended as potential botanicals for the control of ALS disease of common bean Var. GLP 1127 Mwezi moja.

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ACRONYMS, ABBREVIATIONS AND SYMBOLS

ALS: Angular leaf spot

^{o}C: Degrees centigrade

Hrs: Hours

Kg: Kilogram

Kg/ha: Kilogram per hectare

IDM: Integrated Disease Management

MT: Metric ton

Mg: Milligram

ML: Millilitre

Min: Minute

Mm: Millimetre

PDA: Potato dextrose agar

PSI: Pressure per Square Inch

±: Plus, or minus

>: Greater than

≤: Equal or less than

Nm: Nanometre

X: Magnification

PHI: Pre harvest interval

SC: Soluble concentrate

MP: Manufacture's prescription

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CHAPTER ONE

INTRODUCTION

1.1 Background to the study

Common bean (*Phaseolus vulgaris*) is among the most important pulse crops in the world (Leitich *et al*., 2016). It can be grouped into dry and green (snap) bean and in Africa majorly produced in climatic regions of temperate and sub-tropical, among other continents (Pamela *et al*., 2014). According to Damiano *et al*. (2014) common bean is consumed throughout the world and comes second in the order of importance. It is the third most important source of calories and fibre in both Eastern and Southern Africa and rich source of protein, zinc and iron (Bode *et al*., 2017; Keller *et al*., 2015; Pamela *et al*., 2014). Kenya is the seventh biggest producer of common bean globally and the second leading producer in East Africa (KenInvest, 2016). The main producing areas for common bean in Kenya include the Rift Valley, Eastern, Lake Victoria zone, Western and Central regions that account for 33%, 24%, 18%, 13% and 20% respectively of national production. National consumption is assessed to be about 755,000MT annually against a production of about 600,000MT a year. Per capita consumption is estimated at 14 kg per year, but can be as high as 66 kg per year in the country's Western regions (KenInvest, 2016). In Kenya, common bean is largely grown by small scale farmers with fewer than five acres and mostly intercropped with maize. They play an essential role in the sustainable livelihoods of smallholder farmers and their families by providing both food security and income generation (CIAT, 2014; Muui *et al*., 2007). In 2007, world's production of common bean was estimated at 417,00 Metric tomes (Mt) against a growing demand of more than 500,000 Mt, the deficit largely attributed to extreme biophysical stresses such as climate change, pest and phytopathogenic diseases, and soil fertility (Katungi *et al*., 2011). Phytopathogenic diseases make the largest proportion of the stresses leading to constant

production of less than 25% of the potential yield (Ddamulira *et al*., 2014; Mauricio *et al*., 2012).

Fungus *Phaeoisariopsis griseola* is the etiologic agent of angular leaf spot of common bean and very destructive phytopathogen of common bean (Allorent and Savary, 2005). It attacks literally all the aerial plant parts such as stem leaves and pods causing shrivelled pods, sunken seeds and premature defoliation (Allorent and Savary, 2005; Bode *et al*., 2017). Angular leaf spot disease is primarily controlled using synthetic fungicide and though it effective has numerous shortcomings such as high input cost, human and environmental hazards and development of pathogen resistance towards the fungicide (Ddamulira *et al*., 2014). Use of resistant varieties have proved to be expensive in terms of development and maintenance due to abundant genetic variability, virulence and pathotype diversity of the pathogen (Sharma and Adikshita, 2017).

The medicinal and antimicrobial activities of extracts from plants are gaining attention of researchers worldwide (Shabana *et al*., 2017). The modern synthetic fungicide has its own advantages and side effects, so the plant-based products are gaining popularity, as they are safe to use, easily available and affordable. Many plant extracts possess antifungal activities according to Cherkupally *et al*. (2017) who reported *D. stamonium*, *E. globulus*, *A. sativum* and *M. charantia* extracts effectively suppressed radial mycelial growth of *R. solani*. Plant extracts are effective in managing plant pathogens and are now becoming popular throughout the world according to Shabana *et al*. (2017) who reported that *in vitro*-tested plant extracts (neem, clove, *antha mandhaari*, black' cumin, white cedar, Brazilian pepper, garlic and garden quinine) inhibited spore germination of wheat leaf rust *P. triticina* by 93% or more. *Azadirachta indica* (neem) is a medicinal plant of importance with phytochemical compounds such as terpenes and alkaloids in various parts of the plant such as bark, leaves and seeds, and has been reported to display antimicrobial properties against pathogenic organisms such as

fungi, bacteria, viruses and pests (Pankaj *et al*., 2011; Al-hazmi, 2013). *Azadirachta indica* extracts have activity against phyto-pathogenic bacteria and fungi such as *Xanthomonas vesicatoria* and *Ralstonia solanacearum* (Sarawaneeyaruk *et al*., 2015). Plant extracts of Azadirachta *indica* have been reported to be effective in the management of several phytopathogenic diseases though little information has been reported on the use of leaves extracts of *Azadirachta indica* in the management of *Phaeoisariopsis griseola* pathogen of common beans. There is therefore need to determine the potential of neem leaves extracts for fungicidal activity against angular leaf spot disease of common bean. *Allium sativum* commonly known as garlic belongs to the family Alliaceae and among the important earliest known medicinal plants (Stavělíková**,** 2008; Byrappa, 2015). Garlic has alliin as its main chemical component which is an alkyl derivative of cysteine alkyl sulfoxide which is responsible for the characteristic odour of garlic thus an important food spice plant (Singh *et al*., 2015). Its usage worldwide has a long history with significant role in disease prevention and control and has been used since long time against human pathogens such as for the treatment of dysentery and worms in infants and adults (Cecilia and Olubunmi, 2014). However, limited information is available regarding the usage of *Allium sativum* crude extract against *Phaeoisariopsis griseola*. Mexican sunflower (*Tithonia diversifolia*) is a member of Asteraceae family. The plant is usually found growing on the road sides, river banks and uncultivated bare lands and has been used for bruises and wound treatment (Gray *et al*., 2013). It has demonstrated successful antimicrobial activity against food and human pathogens (Rejeki & Addy, 2017). Little information is available on its use for control against plant pathogens. Globally, there is growing demand for natural botanicals among common bean growers for use as bio-fungicides due to the numerous negative effects of the synthetic fungicides on the environment and human health (Cecilia and Olubunmi, 2014). Plant parts of *Azadirachta indica, Allium sativum* and *Tithonia diversifolia* have been widely used in the

control of phyto-pathogens (Rejeki & Addy, 2017). These plants have been reported to have antimicrobial activity against a number of pathogens (Gray *et al*., 2013). Little is known regarding the use of leaves extracts of neem and Mexican sunflower, and garlic as botanicals in the control of angular leaf spot disease of common beans. This therefore, necessitates the need to search for pathogen management options that are environmentally friendly, harmless to the non-target organisms and human health, and keep the pathogen to levels below economic threshold.

1.2 Statement of the problem

Common bean is rank second to maize in order of importance as a food crop. Both maize and common bean are cultivated almost exclusively by about 1.5 million smallholder farmers on about a million hectares, with yields of about 0.6MT/ha. Kenya is the seventh biggest producer of common bean globally and the second leading producer in East Africa (KenInvest, 2016). The main producing areas for common bean in Kenya include the Rift Valley, Eastern, Lake Victoria zone, Western and Central regions that account for 33%, 24%, 18%, 13% and 20% respectively of national production. National consumption is assessed to be about 755,000MT annually against a production of about 600,000MT a year. Per capita consumption is estimated at 14 kg per year, but can be as high as 66 kg per year in the country's Western regions. Common bean production has been declining in Kenya, from 714,492 tons in 2013 to 615,992 tons in 2014 (KenInvest, 2016). In Kenya, common bean is largely grown by small scale farmers with fewer than five acres and mostly intercropped with maize. They play an essential role in the sustainable livelihoods of smallholder farmers and their families by providing both food security and income generation (Muui *et al*., 2007; CIAT, 2014). However, despite the importance, the productivity of maize and bean has remained low at 1-2 t/ha and below 1 t/ha, respectively and unable to meet rising population food demands (Olwande, 2012; Otieno 2019). Such low yields among smallholders who are the main producers in Kenya are largely

due to phytopathogenic diseases especially angular leaf spot of common bean (Fikre *et al*., 2011). Angular Leaf Spot disease is a major biotic constraint of common bean production in Western Kenya with losses as high as 80% under disease favourable environmental conditions (Leitich *et al*., 2016). *Phaeoisariopsis griseola* fungus is the causative agent of angular leaf spot disease. The pathogenic fungus is extremely challenging to manage through chemical, cultural techniques or use of resistant varieties. The use of synthetic fungicide in agricultural farming has a number of shortcomings such as being toxic to non-targeted microbes, development of pathogenic resistance, high input costs to the farmers in the third world countries and hazardous to the environment due to residual effect (Sharma and Adikshita, 2017). Angular leaf spot disease thus poses a chronic threat to food security in Kenya. Lack of elaborate control of angular leaf spot disease has led to yield reduction of 40% -80% (Fikre *et al*., 2011). This calls for the urgent need to identify an eco-friendly, inexpensive and an alternative to the chemical inorganic fungicide that can be used in the control of pathogens. The medicinal and antimicrobial activities of extracts from plants are gaining attention of researchers worldwide. Apart from the use of plant-based products in medicine, the usage of these extracts in plant protection is now becoming popular throughout the world. Little is known about the success of using *Allium sativum, Azadirachta indica* and *Tithonia diversifolia* crude extracts to control *Phaeoisariopsis griseola* of common bean. Synthetic fungicides have been reported to significantly influence common bean growth and yield through disease reduction though limited information is available on the effect of botanicals to influence common bean growth and yield. The aim of the study was therefore to come-up with an alternative angular leaf spot disease management option that was least harmful to both human and environment.

1.3. Justification

The use of synthetic fungicides in agricultural farming has increased consumer concern and has several drawbacks thus its use is becoming more restrictive due to carcinogenic effects, residual toxicity problems on the environment, development of resistance in pathogenic microbes, toxic to non-target microbes and very expensive for farmers in developing countries (Wang *et al*., 2010). In Kenya, for instance, a bigger percentage of common bean production is from medium and small or subsistence farmers who do not apply synthetic fungicides to their crops due to high cost (Leitich *et al*., 2016). The inefficiency of the chemical fungicides in the control of angular leaf spot disease and the high cost for production of a resistant varieties, demands in food production and food security through the use of technology that is not detrimental to humans and the environment is a major challenge to the bean farmers. Due to genetic variability and virulence, the pathogen develops resistance towards the chemical fungicides which in the long-run leads to constant losses that has a significant impact on the production of common beans over seasons. Taking into account the challenges, it has become important to develop strategies based on rational use of the fungicides or to replace them with alternative products. Plant extracts have shown biological activity that may pave way for an alternative option in the management of angular leaf spot disease due to its fungi-toxic action in the plant. Little information about the fungicidal activity of leaves extracts of *Azadirachta indica* and *Tithonia diversifolia,* and *Allium sativum* against angular leaf spot disease of common bean has been reported. Effective control of angular leaf spot of common bean lies on the management options that have the merits of being readily available in farming localities of the tropics, cheap, eco-compatible, less harmful to non-target organisms and useable in integrated disease management programmes for smallholder, resource-poor farmers. Thus, this study aims to screen the selected plant extracts against *Phaeoisariopsis griseola* pathogens to establish its effectiveness as alternatives to synthetic fungicides in the control of angular leaf spot disease of common beans. This study is vital considering that cultural, chemical and use

of resistant varieties control techniques have been employed to manage angular leaf spot disease but huge yield losses are still experienced by bean farmers. This has prompted the need to try out alternative technologies in a bid to develop a suitable control for *Phaeoisariopsis griseola* such as the use of botanicals. The documentation of the effectiveness of the selected plant extracts against angular leaf spot disease of common bean will ultimately provide potential sources of widely sought remedies to the losses incurred in common bean production due to angular leaf spot disease infection. This will lead to isolation of bioactive compounds which will serve as starting materials for laboratory synthesis of biopesticides as well as models for the production of biologically active compounds (Dhanani *et al*., 2017). This study provides a frame work on the possible plant extracts that can serve as source of constituent for the commercial production of remedies against angular leaf spot disease of common bean.

1.4. Objectives of the study

1.4.1. General objective

The general objective of this study was to characterise *Phaeoisariopsis griseola* from infected common bean, phytochemically screen and determine fungicidal activity of *Azadirachta indica, Allium sativum* and *Tithonia diversifolia* plant extracts against *Phaeoisariopsis griseola* causing angular leaf spot disease of common bean Var. GLP 1127 Mwezi moja (Maseno, Kenya).

1.4.2. Specific objectives

1. To morphologically profile isolates of *Phaeoisariopsis griseola* pathogen from infected common bean from Sabatia, Ugenya and Bondo sub-counties.

2. To determine the phytochemical composition of bulbs of *Allium sativum*, and leaves of *Azadirachta indica* and *Tithonia diversifolia.*

3. To determine the effect of *Azadirachta indica, Allium sativum* and *Tithonia diversifolia* crude extracts on spore germination of *Phaeoisariopsis griseola* pathogen obtained using

solvents extracts of distilled water, methanol and ethanol at 50, 75 and 100% extract concentrations.

4. To determine the effects of *Azadirachta indica, Allium sativum* and *Tithonia diversifolia* methanolic crude extracts on the growth and yield components of common bean Var. GLP 1127 Mwezi moja infected with *Phaeoisariopsis griseola*.

5. To determine the effect of *Azadirachta indica, Allium sativum* and *Tithonia diversifolia* methanolic crude extracts on disease incidence and severity of angular leaf spot disease of common bean Var. GLP 1127 Mwezi moja.

1.5 Hypotheses

1. There are morphologically different isolates of *Phaeoisariopsis griseola* pathogen on infected common bean from Sabatia, Ugenya and Bondo sub-counties.

2. There are various phytochemical compounds in *Azadirachta indica, Allium sativum* and *Tithonia diversifolia*.

3. *Azadirachta indica, Allium sativum* and *Tithonia diversifolia* crude extracts have effects on spore germination of *Phaeoisariopsis griseola* pathogen at different extract concentrations.

4. *Azadirachta indica, Allium sativum* and *Tithonia diversifolia* methanolic crude extracts have significant effect on the growth and yield components of common bean Var. GLP 1127 Mwezi moja.

5. *Azadirachta indica, Allium sativum* and *Tithonia diversifolia* methanolic crude extracts have high significant effect on disease incidence and severity of angular leaf spot disease of common bean Var. GLP 1127 Mwezi moja.

CHAPTER TWO

LITERATURE REVIEW

2.1 Common bean (*Phaseolus vulgaris* L.) botanical description

Common bean is an annual herbaceous plant, erect or climbing with leaves composed of rhomboid or tri-oval shaped leaflets (Katungi *et al*., 2009). Common bean varieties can be indeterminate, determinate and matas low or climbing (Creamer, 2014). The erect plant has stems and fickle tendrils which are made by modification of terminal leaflets while sometimes covered by villi (Damiano *et al*., 2013). It is largely a self-pollinated plant though cross-pollination is possible if the stigma contacts with pollen coated bee when extended (Keller *et al*., 2015). Seeds are non-endospermic and vary greatly in size and colour from the small black wild type to the large white, brown, red, black or mottled seeds of cultivars, which are 7-16 mm long (Katungi *et al*., 2009). Common bean is grown for both food and cash value in monoculture and a number of cropping systems in East Africa (Fikre *et al*., 2011). Over 111,000 years ago, common bean has been reported to have diverged from the Mesoamerica and Andes centres of origin which have led to the gene pools Mesoamerican bean and the Andean bean which can be differentiated on seed size, with Andean bean being large seeded while Mesoamerican is small seeded (Keller *et al*., 2015). Common bean has an extensive root system which is made of taproot, tuberous root and several secondary roots and seeds range in colour from yellow, black, grey, brown, white, red, purple and pinto or fluted (Damiano *et al*., 2013). Common bean is a member of family Fabaceae and an annual, self-pollinating pulse crop (legume) grown almost worldwide under variable climatic conditions from tropical to sub-tropical regions (Chilagane *et al*., 2013) Common bean production has been reported to have the potential of reducing food insecurity and poverty levels as the legume serves the primary source of protein and nutrition (Creamer, 2014). Common bean belongs to the genus *Phaseolus* which is composed of more than fifty plant species which are all native to America (Damiano *et al*., 2013). The flowers are asymmetric in shape and purple or white in colour while the fruits

are variable in colour with 3-12 seeds in the fruit (pod) (Damiano *et al*., 2013). Common bean thrives well in warm climatic conditions with an average temperature of 16 - 26^{o}C and rainfall of 300-500,000mm throughout the growing season. The production is by means of seeds (Fikre *et al*., 2011). Generally, common bean is considered a short-season crop with most varieties maturing in a range of 65 to 110 days from emergence to physiological maturing (Katungi *et al*., 2009).

2.2. Taxonomy and classification of common bean

Divisions: Magnoliophyta

Class: Magnoliopsida

Order: Fabales

Family: Fabaceae

Genus: *Phaseolus*

Specie: *Phaseolus vulgaris*

Source: (Damiano *et al*., 2014).

2.3. Common bean varieties in Kenya

Some of the common bean varieties grown in Kenya are Monei, Amy, Samantha, Teresa, Julia, Vernando, Bronco, Coby, Espadia, Bakara, Rose Coco, Mwitemania, Wairimu, Mwezi Moja, Canadian W, KK 15 (Leitich *et al*., 2016).

2.4. Common bean variety GLP 1127 Mwezi moja

GLP 1127 Mwezi moja common bean variety is an early maturing at about 85 days, vigorously growing and determinate variety (Shisanya, 2004). The seeds are large, light brown, oblong beige or speckled purple and are well suited for relatively drier, semi-arid or low precipitated areas also performs well in medium rainfall areas during short rain seasons. GLP 1127 Mwezi moja is high yielding though highly susceptible to angular leaf spot disease but symptoms appear during late cycles of the plant life after flowering. The seeds are preferred since they

cook fast and tastes well. The market requires freshy, straight, long, rounded in cross-section beans qualities found in Mwezi moja (Leitich *et al*., 2016).

2.5. Ecological requirements for growing common beans

Common beans grow well in temperatures ranging from 15-33 degree centigrade. However, an optimum growing temperature of 20-25 degrees centigrade is essential. Relatively high temperatures affect flowering and pod setting processes (Shisanya, 2004). The crop is very sensitive to frost. Temperatures ranging between 14-32°C though extreme temperatures result to poor flower development and poor pod set (Corte *et al*., 2013). The optimum altitude range between 1,000–2,100m above sea level, it however tends to grow and mature faster in low altitude zones and a well distributed medium to high rainfall of about 800-2000mm annually is suitable for the rain fed production. Irrigation should be done if rainfall is inadequate. Excessive rainfall during flowering causes flower abortion and increased disease incidences. Dry weather conditions are needed during harvesting. Common bean crop thrives in a well-drained loam to heavy clay soil which is rich in organic matter, weed free and has an optimum PH of 6.5-7.5. Growth is poor in waterlogged soils (Leitich *et al*., 2016).

2.6. Importance of common bean

In Kenya, common bean is ranked as second most important staple food after maize (Leitich *et al*., 2016). Common bean is consumed daily as part of dietary protein as a vital legume crop for over half a billion people around the world (Corte *et al*., 2013). Almost more than half of the world population uses this grain legume for direct consumption which involves Eastern and Southern Africa where it is cultivated in over four million hectors of agricultural land (Leitich *et al*., 2016). Common bean as a legume crop plays a vital role in daily diet as it provides carbohydrates, proteins, vitamins and essential elements to both the urban and rural populations (Namugwanya *et al*. 2014). The crop is estimated to supply more than half of the dietary protein required by households in Africa (Tryphone *et al*., 2016). Common bean represents one of the principal crops in East Africa in terms of total area planted and number of farmers involved in

production. Common bean production also provides farm households with both source of income and food for nutrition through sales and consumption of part of the produce (Anderson, 2010). Consuming beans also have medicinal benefits as it is recognized that they contribute to treating human ailments like cancer, diabetes, and heart diseases (Bode *et al*., 2017). Evidence points out that poverty levels would have been higher in the absence of development and adoption of improved bean varieties (Leitich *et al*., 2016).

2.7. Common bean production

Common bean yields in Kenya have remained low with an average yield of 585 kg/ha compared to Ethiopia and Rwanda with yields of 1588 kg/ha and 913 kg/ha respectively (Leitich *et al*., 2016). Globally, common bean is cultivated on about 28 million ha, producing on average, approximately 715 kg year-1 ha-1 (Namugwanya *et al*., 2014). According to Katungi *et al*. (2011) production in 2007 was about 417,000 metric tons while demand was estimated at 500,000 metric ton. The supply deficit is attributed to the severity of biophysical stresses such as climatic variability, insect pests and diseases and declined soil fertility that maintain productivity at less than 25% of potential yield. Plant diseases are the major factor the contribute to low yields of common bean due to lack of effective control mechanism. Further, Leitich *et al*. (2016) reported that in Kenya, per capita consumption is estimated at 14 kg per year, but can be as high as 66 kg per year in Western Kenya. To counter this production menace alternative control needs to be developed to boost the production to acceptable quantities.

2.8. Constraints of common bean production

According to the average yields of common bean has remained low as >500 kg/ha while the potential of current promising released varieties is at 1500 kg/ha (Tryphone *et al*., 2013). In Kenya, common bean yield has constantly remained low (Leitich *et al*., 2016). Angular leaf spot disease is basically the most important constraint to common bean production in Africa with annual loss estimated at 374,800MT (Fikre *et al*., 2011). Across farming systems, biotic

and abiotic stresses continue to present the major constraints for increased bean production and high yields with bean diseases representing the major constraints to production by reducing yields and seed quality. Angular leaf spot disease of common bean can cause yield loss up to 100% of the expected yield, depending on the environment and the varieties used (Fikre *et al*., 2011). The major diseases affecting bean production in East Africa including Kenya are Bean Common Mosaic Necrosis Virus, common bacterial blight (*Xanthomonas axonopodis pv. phaseoli*), angular leaf spot (*Phaeoisariopsis griseola*), anthracnose (*Colletotricum lindemuthianum*) and rust (*Uromyces phaseoli*) (Bode *et al*., 2017). Angular leaf spot is the most destructive of the diseases of common bean in Western Kenya (Leitich *et al*., 2016).

2.9. Angular leaf spot disease of common bean

Angular leafspot caused by *P. griseola* attacks only common beans (*Phaseolus vulgaris* L.) and lima beans (*Phaseolus lunatus* L.) (Mongi, 2016). Pathogenic fungi are the main infectious agents in plants, causing alterations during developmental stages including post-harvest. Angular leaf spot disease of common bean (*Phaseolus vulgaris* L.), caused by the fungus *Phaeoisariopsis griseola* a plant pathogenic fungus (Sartorato, 2002). Angular leaf spot is among the most destructive diseases of common bean particularly in relatively warm, humid, areas with abundant inoculum from infected volunteer plants, infected plant debris, off-season crops and contaminated seeds (Ddamulira *et al*., 2014; Fikre *et al*., 2011). The disease is ranked second among biotic and abiotic factors that constrain bean production in Africa. It is also one of the most widely distributed and damaging diseases of common bean (*Phaseolus vulgaris* L) in tropical and subtropical countries with yield losses on susceptible varieties can be as high as 80%. The infected host exhibits reduced photosynthetic rate due to abnormalities in form and function of chloroplasts of the diseased tissue followed by decline in photophosphorylation, photochemical reaction and carbon dioxide assimilation reducing physiological performance of the canopy (Mongi, 2016; Sharma and Adikshita, 2017).

2.9.1. Taxonomy and diversity of Angular leaf spot (*Phaeoisariopsis griseola*)

Angular leaf spot disease is found in more than sixty countries throughout the world (Sartorato, 2002). In Kenya, forty-four physiological races of *P. griseola* have so far been identified. *Phaeoisariopsis griseola* virulence is assessed based on reaction of isolates on a standard differential set of 12 common bean varieties proposed by CIAT, and divided into two sets of Mesoamerican and Andean, with six varieties each (Leitich *et al*., 2016). The pathogen has been a big challenge to local farmers though little is known of *Phaeoisariopsis griseola* in sub-counties of Sabatia, Bondo and Ugenya and correct pathogenicity test could lead to effective disease management.

2.9.2 Life cycle, epidemiology and dissemination of angular leaf spot disease

Phaeoisariopsis griseola spores germinate on the leaf surface after 3 days of moist conditions, enter the leaf through the stomata and grow inter-cellularly, limited by the leaf veins resulting in an angular lesion shape (Oblessu *et al*., 2015). Germination of fungal spores is essentially a process during which the normal metabolic and physiological activity is restored after dormancy which involves spore transformation from a dormant state of low metabolic activity done of high metabolic activities (Singh *et al*., 2014). Infection and sporulation occur in a broad temperature range, from 10 to 33°C (Keller *et al*., 2015). The disease is favoured by intermittent dry-wet and warm-cool weather (Sartorato, 2002). Angular Leaf Spot epidemics are usually observed relatively late in the crop cycle typically about the flowering stage. *P. griseola* spore germination and hypha-penetration is through the epidermis or stomata as illustrated in Fig. 2.1 (Allorent and Savary, 2005). The hemi-biotrophic life cycle of this fungus comprises of intercellular hyphae growth in the plant leaf mesophyll during the biotrophic phase, and sub-sequent hyphae penetration of the host cell causing plasmolysis during the fungus necrotrophic phase on susceptible plant genotypes (Oblessu *et al*., 2015). Little information is available on the effects of plant extracts on fungal spore germination.

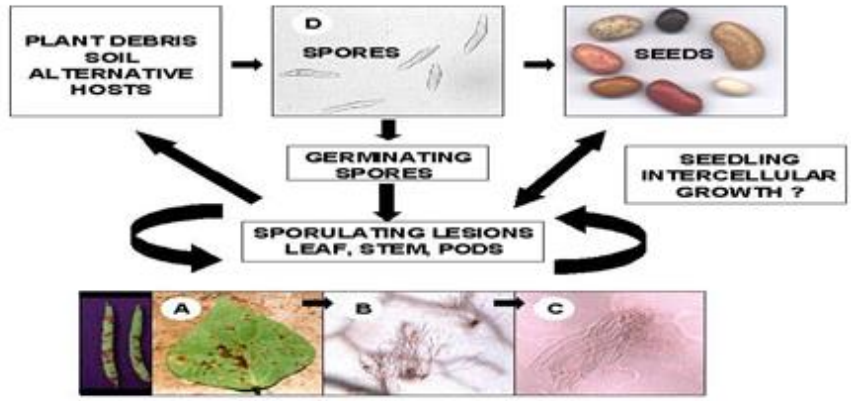


Figure 2.1: Angular leaf spot disease cycle. Courtesy of Allorent and Savary (2005).

2.9.3. Symptomatology of Angular leaf spot disease of common bean

Symptoms induced by *P. griseola* on beans develop on leaves, stems and pods. Lesions can appear on primary leaves within 6 days after inoculation, but usually do not become prevalent until the late flowering or early pod-set stage (Ddamulira *et al*., 2014). The spots are small, angular, dark brown and often so numerous that they give the foliage a checkerboard appearance (Sartorato, 2002). Lesions may increase in size, coalesce and cause necrosis and yellowing. When *P. griseola* attacks a plant, spots originate on the underside of the leaf and are delimited by the veins and veinlets. At first, the lesions are grey, later turn brown and attain an angular shape because of limitation by veins (Allorent and Savary, 2005). Pod lesions are roughly circular and reddish brown with dark brown borders (Sartorato, 2002). Stem lesions are dark brown and elongate (Ddamulira *et al*., 2014). On all lesions, dark stroma appears in abundance. Angular leaf spot disease causes typical symptoms on the leaves with angular shaped lesions as well as lesion multiplication and extension on the foliage lead to defoliation, a prime mechanism leading to reduced physiological performance of plant cover (Allorent and Savary, 2005). Furthermore, late infection on pods and seeds, also cause scars that reduce on seed quality and market value (Ddamulira *et al*., 2014). Lesions on other aerial plant parts like

stems, petioles, and pods (Plates 2.1, 2.2 and 2.3). Angular leaf spot epidemics is majorly observed relatively late in the crop cycle typically the flowering stage (Allorent and Savary, 2005). Effective pathogen identification is anchored on symptomatology by carrying out pathogenicity tests. Pathogenicity test is carried out to establish whether the fungal isolates cause the disease and to determine whether they could induce similar symptoms on inoculation and be re-isolated, thus fulfilling Koch's postulates (Ezeonu *et al*., 2018). There is scanty information on the pathogenicity of the isolates of *P. griseola* fungus.

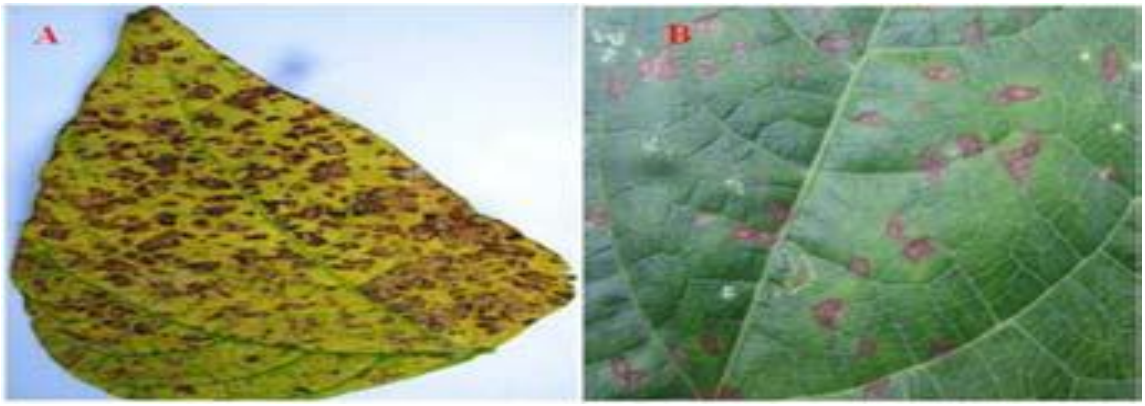


Plate 2.1: Leaf A and B infected by angular leaf spot disease. Courtesy of Allorent and Savary (2005).

Plate 2.2: Angular leaf spot disease causing severe leaf defoliation. Courtesy of Allorent and Savary (2005).

Plate 2.3: common bean pod attacked by angular leaf spot disease. Courtesy of Ddamulira *et al*. (2014).

2.9.4 Effect of angular leaf spot on growth and yield component of common bean

Late infection on pods and seeds causes scars that reduce seed quality and market value (Ddamulira *et al*., 2014). Angular leaf spot disease is basically the most important constrain to common bean production in Africa with annual loss estimated at 374,800MT (Fikre *et al*., 2011). Across farming systems, biotic and abiotic stresses continue to present the major constraints for increased bean production and high yields with bean diseases representing the major constraints to production by reducing yields and seed quality (Ddamulira *et al*., 2014). They can cause yield loss up to 100% of the expected yield, depending on the environment and the varieties used. Pathogenic fungi are the main infectious agents in plants, causing alterations during developmental stages including post-harvest. There are very few studies on the effect of angular leaf spot disease on common bean growth and yield component.

2.9.5. Control of angular leaf spot disease

Angular leaf spot disease of common bean is managed through cultural practices such as crop rotation and cultivar mixtures (Sharma and Adikshita, 2017). However, these have limited potential in managing the disease, because land scarcity cannot allow crop rotation to be practiced. Moreover, effective methods of ALS control like use of fungicide are far beyond the means of low resource endowered farmers. This is because of the high cost and long-term

consequences fungicide pose to the environment (Ddamulira *et al*., 2014). Angular leaf spot can be more efficiently controlled through fungicide sprays and resistant varieties while the use of genetic resistance is the most appropriate, safe and cost-effective way to control ALS among smallholder farmers (Sartorato, 2002; Ddamulira *et al*., 2014). The use of resistance varieties is highly recommended but according to Sharma and Adikshita (2017) host resistance is difficult to maintain because the abundant virulence and pathotype diversity of *Phaeiosariopsis griseola* renders varieties that are resistant in one location or year susceptible in another. A study by Sharma and Adikshita (2017) reveals that seed treatment and foliar spray with carbendazim and mancozeb either alone or in combination is frequently used for the management of angular leaf spot of beans. But continuous use of these fungicides could result in development of resistance strains and farmers are reporting the ineffectiveness of these fungicides (Sartorato, 2002). The use of fungicides in angular leaf spot disease control has been elaborately studied though little has been reported on the effect plant extracts for the control of angular leaf spot disease especially crude extracts of *Azadirachta indica, Tithonia diversifolia* and *Allium sativum*.

2.10 Plant extracts

Phytochemical study of plant is important for modern day agriculture but its usefulness cannot be overemphasized if methods are not standardized to obtain comparable and reproducible results. Plants naturally synthesize diverse group of secondary metabolites which constitute an important source of microbicides, pesticides and many pharmaceutical drugs (Shabana *et al*., 2017). In these natural sources, a series of molecules with antifungal activity against different strains of fungus have been found, which have great importance to human and plants (Cherkupally *et al*., 2017). Plants perform as a renewable natural resource of diverse bioactive compounds (Ramadass & Subramanian, 2018). Plant extracts consist of various compounds characteristic to the plant from which they were extracted. Aromatic secondary metabolites synthesized by plants are phenols, phenolic acids, quinones, flavones, flavonoids, flavonols,

tannins and coumarins. Compounds with phenolic structure have high efficiency against plant pathogens because of their antimicrobial activity (Shabana *et al*., 2017). Plants have been proved as useful source of several antifungal molecules that are harmless and caring to the environment (Ramadass & Subramanian, 2018). Obongoya *et al.* (2010) reported that *Azadirachta indica* had reduced disease incidence of *Fusarium oxysporum* of common bean by about 17% while *Tagetes minuta*, *Nicotiana tobacum* and *Inca rosea* had a reduction range of 5.84-9.8%. Analysis of variance at 95% showed that all the parts (leaf, bark and seed) of the test plant (neem) used significantly ($P < 0.05$) inhibited the growth of the fungal organisms against cocoyam rot (Ezeonu *et al*., 2018). The use of botanicals for control of foliar diseases have gained importance due to the recent global awareness negative effect of chemical fungicides, such as development of resistance, associated resurgence in fungi, accumulation of fungicide residues in food chain, environmental pollution, health risks and high costs (Ramadass & Subramanian, 2018). There are certain advantages in the deployment of botanical pesticides. These are biodegradable, safe to non-target organisms, renewable and suit to sustainability of local ecology and environment (Cherkupally *et al*., 2017). This has awakened new interest in natural products as a source for novel industrial plant protection strategies. It would be advantageous not only to standardize methods of extraction or to test the *in vitro* antimicrobial efficacy; but the crude extracts or the discovered compounds should be subjected to *in vivo* testing to evaluate the efficacy in controlling the incidence of disease in crops through pot or field experiments (Ramadass & Subramanian, 2018). It is therefore essential to carry out the complete development of an interesting lead compound into an exploitable product.

2.11. Synthetic chemicals

The adoption of chemicals has had an undeniable benefit for modern agriculture; the yields increase has been significant because of the discovery and application of synthetic measures for pest control (Dayan *et al*., 2009). Due to the continuously growing agronomy sector, there

is a growing demand for fungicides and pesticides because of new and existing plant diseases, which could have an adverse impact on yields. Conventional chemicals applied in agriculture for plant disease control such as benzimidazoles, aromatic hydrocarbons and sterol biosynthesis inhibitors are related to various problems (Stevic *et al*., 2014); it is already known that chemical fungicides and pesticides have negative effects on the environment and human health. Plant pathogens develop or have already acquired resistance to widely used chemical products. Plant growers feel a responsibility to reduce the application of pesticides as their residues could be found in their produce (Cherkupally *et al*., 2017).

2.12. Neem plant botanical and taxonomic description

Neem is a tropical evergreen tree and of two species of *Azadirachta* genus of *Meliaceae* family, *Azadirachta indica* native to India and *Azadirachta excelsa* found in Indonesia and Philippines (Pankaj *et al*., 2011; Ravishankar *et al.*, 2018). Native to Indian sub-continent, *Azadirachta indica* is a member of the Mahogany family and has similar properties to its relative, *Melia azedarach* (Pankaj *et al*., 2011). It is a speedy growing tree which can grow up to 30 meters tall with an average girth of 2.5meters (Ravishankar *et al.*, 2018).

The taxonomic positions of neem are as follows:

Order: Rutales

Family: Meliaceae

Genus: *Azadirachta*

Species: *indica*

Latin: *Azadirachta indica*

Source: (Pankaj *et al*., 2011).

2.12.1 Uses of *Azadirachta indica*

In a document by Ahmed (2008) the seeds, bark and leaves contain compounds with proven antiseptic, antiviral, antipyretic, anti-inflammatory, anti-ulcer and antifungal use. *Azadirachta indica* is reported to be a natural source of eco-friendly insecticides, pesticides and

agrochemicals. Leaf extracts of *Azadirachta indica* have been reported to be effective in elimination of *Fusarium spp* of African yam bean, brinjal and tomato (Obongoya *et al*., 2010). Alcoholic extracts of *Azadirachta indica* have high inhibitory effect on fungus; the fungus targeted were *Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger* and *Aspergillus terreus* (Ravishankar *et al.*, 2018). To date there has been little study of *Azadirachta indica* leaves extracts for fungicidal activity against angular leaf spot disease of common bean.

2.12.2. Phytochemical compounds of *Azadirachta indica* plant

A large number of bioactive secondary metabolites, such as coumarins, alkaloids, limonoids and some essential oils have been isolated from different plant parts of neem plant (Shabana *et al*., 2017). There are around 135 phytocompounds that have been isolated from different parts of neem tree (Ravishankar *et al.*, 2018). These phytocompounds can be divided into two different categories as shown in Table 2.1(Ravishankar *et al.*, 2018). Sulphur containing compounds such as trisulphide and tetra-sulphide components of *Azadirachta indica* leaves have antifungal activity against *Trichophyton mentagrophytes* (Pankaj *et al*., 2011). The chemical constituents contain many biologically active compounds that can be extracted from neem, including alkaloids, flavonoids, triterpenoids, phenolic compounds, Carotenoids, steroids and ketones (Ahmed, 2008)

Table 2.1: Types of phytocompounds found in the various parts of neem plant

Isoprenoids	**Non- isoprenoids**
Diterpenoids and triterpenoids containing protomeliacins	Proteins (amino acods)
Limonoids (meliantrol)	Carbohydrates(polysaccharides)
Azadirone and its derivatives	Sulphurous compounds
Gedunin and its derivatives	Polyphenolics and their glycosides
Vilasinin type of compounds	Dihydrochalcone, coumarin
C- secomeliacins such as nimbin, salanin and azadirachtin	Tannins and aliphatic compounds.

Source: (Ahmed, 2008).

2.13. Taxonomy and biology of *Allium sativum*

Garlic is a member of Liliaceae family having botanical name, garlic (*Allium sativum)* is a common vegetable used widely in almost all parts of the world (Byrappa, 2015; Stavělíková**,** 2008). Originally from Central Asia, garlic is one of the earliest cultivated plants (Bhandari, 2012). The taxonomic positions of garlic are as follows:

Class: Liliopsida,

Superorder: Liliianae,

Order: Amaryllidales,

Family: Alliaceae,

Genus: *Allium*

Specie: *sativum*

Source: (Stavělíková, 2008).

There are several varieties of garlic but the most common varieties are white and pink garlic (Cecilia and Olubunmi, 2014). The main chemical constituent of intact garlic is the amino acid "alliin". It is an alkyl derivative of cysteine alkyl sulfoxide, responsible for the typical odour (Singh *et al*., 2015). Allicin is believed to be the natural chemical component that is responsible for antimicrobial effects of garlic (Baljeet *et al*., 2015). Byrappa (2015) stated that, family Liliaceae has more than 500 members which can be distinguished morphologically, by colour and taste though their neutral-ceutical and phytochemical composition are close. For example, extract of *Allium sativum* has antimicrobial properties because of secondary metabolites from amino acids, produced by hydrolysis (Cherkupally *et al*., 2017).

2.13.1 Uses of *Allium sativum*

Allium sativum has been used in the treatment of worms and dysentery in children and adults. For cooking it is used for seasoning (Cecilia and Olubunmi, 2014). Allium vegetables,

particularly garlic exhibit a broad antibiotic spectrum against both Gram-positive and Gram-negative bacteria such as *Escherichia coli, salmonella, staphylococcus* and *streptococcus* species and against the fungal pathogens such as *Botrytis cinerea, Pythium ultimum* and *Rhizoctonia solani* (Lindsey and Staden, 2004, Stavělíková**,** 2008). The inhibitory effect of garlic is depended on its concentration (Baljeet *et al.,* 2015). Garlic has been used for hundreds of years to treat even fungal, parasitic and viral infections (Lindsey and Staden, 2004). *Allium sativum* is known for having an array of antifungal, antiviral and antibacterial properties (Baljeet *et al.,* 2015).

2.13.2 Phytochemical compounds of garlic

Garlic contains more than 200 chemical compounds with at least 33 sulphur compounds, several enzymes, 17 amino acids and minerals such as selenium (Bhandari, 2012). Garlic's pungent smell and odour and many of its antimicrobial effects are due to the sulphur compounds (Bhandari, 2012). Table 2.2 shows the phytochemical compounds found in garlic.

Table 2.2: Phytochemical constituents of *Allium sativum*

Phytochemical compound	**Observations**
Carbohydrates	+
Proteins	+
Amino acids	+
Volatile oil	+
Saponins	+
Terpenoids	+
Steroids	+
Enzymes	+

Key: + indicates presence - absence

Source: Bhandari, (2012).

2.14. Taxonomy and biology of *Tithonia diversifolia*

Family: Asteraceae

Synonym: *Mirasolia diversifolia* Hemsl.

Vernacular/ common names:

English: Mexican sunflower, *Tithonia*, tree marigold.

Kisii: Amaua maroro.

Luo: Maua makech, akech, maua madungo.

Luhya: Maua amalulu

Source: (Achieng' *et al*., 2010).

Tithonia diversifolia is a woody herb or succulent shrub, 1.2-3 m tall. Opposite leaves 3-5, attenuate base, acute apex and crenate margin. Leaf size is 5-17 x 5-12 cm, densely pubescent beneath and palmate venation. Occasionally upper leaves are unloaded (Ragasa *et al*., 2014). Flowers are yellow; their ray size is 306 cm x 5-18 mm (Plate 5). The flower heads are solitary on a peduncle 6-13 cm long as illustrated in plate 5. Each mature stem may bear several flowers at the top of branches. The plant flowers and produces seeds throughout the year. The light weight seeds can be dispersed by wind, water and animals (Anjarwalla & Jamnadass, 2013).

Plate 2.4: *Tithonia diversifolia* plant with yellow flowers (Achieng' *et al*., 2010).

2.14.1 *Uses of Tithonia diversifolia*

Tithonia diversifolia biomass has potential for soil fertility improvement among smallholder farmers (Achieng' *et al.*, 2010). Pesticidal properties of *Tithonia* spp. are well known for sesquiterpene such as lactones and diterpenoids, some of which have biological activities against insects. *Tithonia diversifolia* has been reported to control fungi *Phytophthora nicotianae* (Rejeki & Addy, 2017). Chloroform extracts and methanol extracts of *Tithonia diversofolia* have inhibitory effect on *Salmonella typhi* and *Staphylococcus* (Ogundare, 2007) Most bioassays have been conducted using extracts so it's not specific about which compounds are responsible for effects. In Uganda, farmers use it in field and storage pest management although there is no published work to report evidence for these effects and /or its use in the management of angular leaf spot disease.

2.14.2. Phytochemical compounds of *Tithonia diversifolia*

Phytochemical analyses of *Tithonia diversifolia* established that flavonoids and tannins are in small concentrations though alkaloids are found in higher concentrations which are bioactive compound that have antimicrobial activity (Ogundare, 2007; Rejeki & Addy, 2017). These bioactive compounds are usually found in high amounts in storage organs of the plants such as stems, bark, root and leaves (Ogundare, 2007). According to finding of Onaran & Saglam (2016) the plant extracts have showed a different level of antifungal activities in a dose depend manner. Substantial use of chemical pesticides induces problems of health and environmental hazards in agricultural system. So, for plants natural products of antimicrobial activity are best bio-rational alternatives today (Das *et al*., 2010).

2.15. Solvent extraction

The initial step in the process of screening medicinal plants for antifungal activity is extraction. Extraction is the separation of biologically active portions of plant tissues using selective solvents through standard procedures. Such extraction techniques separate the soluble plant metabolites and leave behind the insoluble cellular mass (Sasidharan *et al*., 2012). Extraction

is an important step in the itinerary of phyto-chemical processing for the discovery of bioactive constituents from plant materials (Dhanani *et al*., 2017). Most extraction methods involve collection and authentication of plant material, drying, size reduction, extraction, filtration, concentration, drying and reconstruction (Gupta *et al*., 2012). Selection of a suitable extraction technique is also important for the standardization of herbal products as it is utilized in the removal of desirable soluble constituents, leaving out those not required with the aid of the solvents (Dhanani *et al*., 2017). Differences in the structure of phenolic compounds also determine their solubility in solvents of different polarity. Therefore, type of extraction solvent as well as the isolation procedures may have a significant impact on the yield of extraction polyphenols from plants material. According to Dhanani *et al*. (2017) selection of suitable extraction process and optimization of various parameters are critical for up-scaling purposes. Extraction efficiency of various techniques are aimed at achieving high efficacy and efficiency (Gupta *et al*., 2012). Various extraction techniques most commonly used include conventional techniques such as maceration, percolation, infusion, decoction and hot continuous extraction. Recently, alternative methods like ultrasound assisted solvent extraction (UASE), microwave assisted solvent extraction (MASE) and supercritical fluid extractions (SFE) have gained increasing interest during the last three decades (Dhanani *et al*., 2017). currently, the open microwave assisted extraction has been reported to be the simplest, convenient, and most rapid technique for extraction of thermolabile phytoconstituents (Gupta *et al*., 2012).

2.16 Choice of solvents

According to Das *et al*. (2010) successful determination of biologically active compound from plant material is largely dependent on the type of solvent used in the extraction procedure. Properties of a good solvent in plant extractions include low toxicity, ease of evaporation at low heat, promotion of rapid physiologic absorption of the extract, preservative action and inability to cause the extract to complex or dissociate. Gupta *et al*. (2012) indicated that water,

ethanol, methanol, chloroform, methylene dichloride and acetone have been used to isolate antimicrobial compounds from plants. Similarly, Das *et al*. (2010) reported that since nearly all of the identified antimicrobial compounds from plants are aromatic or saturated organic compounds, they are most often obtained through initial ethanol or methanol extraction. Thus, the most commonly used solvents for preliminary investigations of antimicrobial activity in plants are methanol, ethanol and water. A correct choice of solvent is fundamental for obtaining an optimal extraction process. When selecting a solvent consideration should be given to the interaction of the solvent with the matrix and the analyte solubility in the solvent. Preferably the solvent should have a high selectivity towards the analyte of interest and exclude unwanted matrix components (Gupta *et al*., 2012). Thus, the solvent used for the extraction of bioactive compounds must be critically chosen because it will influence the quantity and quality of the final extract (Syukriah *et al*., 2014). According to Anwar and Przybylski, (2012) polar solvents such as methanol and ethanol, either in their aqueous mixtures, are mostly recommended for the extraction of phenolics from a plant matrix. However, according to Sultana *et al.* (2009) aqueous methanol was found to be more effective in recovering highest amounts of phenolic compounds from rice bran and *Moringa oleifera* leaves.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study site

The study was carried out in Maseno University at the Botany laboratory (-0.002358,34.609768) and the greenhouse (-0.004019,34.596085). Maseno University is situated in Kisumu County in Western Kenya with geographical co-ordinates as 0^0 00′ 17″South 34^0 36′ 02″ East with an elevation of 1,503 meters or 4,934 feet above sea level (KNBS, 2013). The climate of Maseno region is tropical with an average temperature of 20.6^{0}C and receives an annual total rainfall of about 1820mm (KNBS, 2013).

3.2 Collection and preservation of *Allium sativum, Azadirachta indica* and *Tithonia diversifolia*

Ten kilograms of fresh and disease-free *Allium sativum* bulbs was purchased from Kibuye market in Kisumu county while disease-free and healthy *Azadirachta indica* and *Tithonia diversifolia* leaves were collected by hand from the Maseno University botanic garden and washed under running tap water to eliminate dust and other foreign particles according to a method by Ezeonu *et al*. (2018). The plant specimens were identified by plant herbarium at the Department of Botany and taxonomic authentication performed by taxonomist at the Department of Botany–Herbarium, Maseno University, Kenya. *Allium sativum* bulbs were split, washed and sliced to await the drying process according to a method of Baljeet *et al*. (2015). The bulbs of *Allium sativum*, leaves of both *Azadirachta indica* and *Tithonia diversifolia* were used in the experiment due to the fact that they contain high levels of active ingredient needed for pathogen control (Obongoya *et al*., 2010; Baljeet *et al*., 2015). Voucher specimens were labelled and brought to the laboratory then stored in the refrigerator at 4°C till use.

3.3 Collection of *Phaeoisariopsis griseola* fungus from Sabatia, Ugenya and Bondo regions

Common bean plants were sampled according to preference from the farmers' fields based on the typical symptoms of angular leaf spot disease of common bean. The aerial parts of the plant especially the leaves were checked for small, dark brown and numerous spots with angular shape limited by the veins. The diseased leaves were collected from three bean growing ecological zones of upper midland zone 1 of Sabatia sub-county in Vihiga county (0^0 31′ 00″North 34^0 34′ 30″ East), lower midland zone 3 of Ugenya sub-county in Siaya county (0^0 10′ 56.2944″North 34^0 17′ 47.9688″ East and low midland zone 4 of Bondo sub-county in Siaya county, 0^0 09′ 73″South 34^0 27′ 64″ East. The sampled diseased plant parts were then zip locked in airtight polyethene bags and placed in ice cooler boxes before they were transported to the laboratory. The collected leaf samples were washed using tap running water and placed in 2% sodium hypochlorite for 2 minutes then blotted by arranging in between absorbent newspapers and pressed carefully to absorb the moisture and distribute pressure evenly across the samples to keep them intact to avoid any breakage (Leitich *et al*., 2016). The samples were stored at 4 ºC to await isolation.

3.4 Sterilization of materials

All glass wares used in this study were washed with detergent, rinsed and sterilized in a dry, ventilated oven at 160°C for 2 h. All media were sterilized by autoclaving at a temperature of 121°C and 15 PSI for 20 min. The scalpel and inoculating needle were sterilized by dipping them into 70% ethanol and passing them over a Bunsen burner flame until red hot according to a method by Ezeonu *et al*. (2018).

3.5 Preparation of culture medium

Throughout the *in vitro* experiment, the assayed culture medium employed was LAB M Potato Dextrose Agar (PDA). This medium was used for the growth and maintenance of the fungal isolates. The preparation of Potato Dextrose Agar (PDA) was done according to the manufacturer recipe. The medium was sterilized by autoclaving at 121 ℃ and 15 PSI for

20 min for complete dissolution and homogeneity. Thereafter, it was allowed to cool to a temperature of 42 °C. One capsule of 323.13 g/mol chloramphenicol was added to every 500 ml of sterile cooled PDA so as to prevent bacteria growth. Approximately 15 ml of the cooled amended PDA was poured into each sterile petri dish of 86 mm diameter to solidify. The petri dishes that contained the medium were incubated for 24 h at 28 °C to check for sterility before use as described by Ezeonu *et al*. (2018). The pathogenicity tests were carried out to establish which of the fungal isolates caused the angular leaf spot and to determine whether they could induce similar symptoms on inoculation and be re-isolated, thus fulfilling Koch's postulates (Ezeonu *et al*., 2018).

3.6 Isolation of *Phaeoisariopsis griseola* and inoculum preparation

Diseased leaves obtained from the different localities received similar treatments separately where they were rinsed in three changes of sterile distilled water. Small sections of the common bean leaf tissue showing small, angular and dark brown spots on the leaves adjoining healthy tissue were cut using sterilized scalpel and whose surface was sterilized with 70% ethanol (Leitich *et al*., 2016). The cut portions were plated out on solidified PDA. Three cut portions were placed per plate with equal distance between them. Four replicate plates for each of the cut portions were made for each of the isolates. The plates were incubated at 24^{o}C for 7 days in a non-illuminated incubator for germination and sub-cultured immediately until a pure culture of the isolates were obtained and stored in McCartney bottles for further use. Sub culturing of the isolates was done to obtain pure culture. The colonies growing on the plates were identified macroscopically and microscopically (Leitich *et al*., 2016). Direct observation of culture under the light microscope (low power) by careful preparation of slides, staining with cotton blue-in-lactophenol was done according to Ezeonu *et al*. (2018). Identification was done using manuals and guides (Alexopoulos, 1962). Spores from the plates were obtained by adding sterile water and transferred onto potato dextrose agar to obtain cultures of the fungus. Inoculating plates were incubated at 24°C for 14 days.

3.7 Morphological profiling of Phaeiosariopsis griseola isolates from Sabatia, Ugenya and Bondo regions

3.7.1 Cultural characteristics

The fungal suspensions of Sabatia, Ugenya and Bondo samples were separately isolated and purified on Potato Dextrose Agar (PDA) to obtain pure culture then inoculated on PDA media to study culture appearance. Cultural characteristics (surface and reverse) were assessed after 14 days on PDA at 25 °C in the dark according to Leitich *et al*. (2016).

3.7.2 Lactophenol blue staining (mycelial morphology)

Mycelial morphology was monitored by light microscopy. Thin smears for Sabatia, Ugenya and Bondo sample plates were prepared separately, heat fixed then flooded with lactophenol blue reagent for one minute. The stain was washed off with tap water and the slides allowed to air dry before being examined microscopically under x400 magnification to reveal microscopic characteristics of the fungus which were assessed after 14 days on PDA at 25 °C in the dark (Crous *et al*., 2006).

3.8 Pathogenicity tests

Considering the importance of correct identification of plant pathogens, pathogenicity tests were carried out to establish whether the fungal isolates caused angular leaf spot disease and to determine whether they could induce similar symptoms on inoculation and be re-isolated, thus fulfilling Koch's postulates (Ezeonu *et al*., 2018). Sabatia, Bondo and Ugenya isolates of *Phaeiosariopsis griseola* causing angular leaf spot disease in common bean was identified following pathogenicity approach. Pathogenicity tests of Sabatia, Bondo and Ugenya isolates was carried out on common bean plants. Common bean seeds were planted in ten plastic pots containing 6kg of solarised soil and watered twice daily until after the tri-foliate leaf was fully formed. The fungal inoculum of 1ml from PDA media was serially diluted and using a hemocytometer about 5 millilitres of 10^{-4} suspension of the fungus inoculum was sprayed into the underside of three different leaves per pot using hypodermic syringe without a needle. Five

millilitres of sterile water were also infiltrated in some common bean plants as controls and left for 48hrs to test for the virulence of the fungus organism according to Emitaro *et al*. (2017). Plants were placed in the greenhouse and observed daily. The inoculated and control plants were covered in plastic bags to maintain humidity at its maximum (Narasimha and Srinivas, 2012). Each experiment was repeated twice, with two replications of 10 plants per treatment. Observations were made from one week after inoculation. The pathogens were re-isolated as previously described and their cultural and morphological characteristics were compared with those of the original isolates.

3.9 Phytochemical screening of *Allium sativum*, *Azadirachta indica* and *Tithonia diversifolia*

The plant parts screened were collected from the Botanical Garden, Maseno University and Kibuye market, identified taxonomically with a taxonomist at the Department of Botany, Maseno university. The plant parts were thoroughly washed then dried under shade until optimum dryness and then pulverised using the lab mill. The final product was then kept inside tight paper bags to await phytochemical screening. *Allium sativum, Azadirachta indica* and *Tithonia diversifolia* were evaluated for the presence of alkaloids, flavonoids, sterols, cardiac glycosides, saponins, tannins and terpenoids using standard procedures.

3.9.1. Test for tannins

Ferric chloride's Test

Half a gram of dried powder of the selected plant parts were added to 20ml of water in a test tube and boiled for 2 min then filtered using Whatman filter paper to obtain filtrate. A drop of 0.1% ferric chloride was added to the filtrate and an intense green colour developed was taken as an evidence for the presence of tannins (Narayana *et al*., 2018).

3.9.2. Test for saponins

Froth test

Two grams of powdered plant parts was mixed with 20ml of distilled water in a boiling tube then place in a hot water bath for 5 min. Filtrate was obtained by using Whatman filter paper. Ten millilitres of the filtrate were mixed with distilled water and the formation of foam indicated the presence of saponins (Emasushan & John, 2018).

3.9.3. Test for flavonoids

Alkaline reagent test

A boiling tube of 5g of powdered plant samples with 10mls of ethyl acetate was heated on steam bath for 3 minutes then filtered. 4mls of the filtrate was shaken with 1ml of dilute ammonium solution and formation of an intense yellow colour which turns to colourless indicated the presence of flavonoids according to method of Susan *et al*. (2018).

3.9.4 Test for terpenoids

Salkowski's test

Five grams of powdered plant samples was placed in test tubes, and 2mls of chloroform added then 3mls of 0.1M sulphuric acid and the formation of a reddish-brown colouration showed positive results for the presence of terpenoids (Parithra *et al*., 2017).

3.9.5 Test for sterols

Salkowski's test

Five grams of powdered plant samples was be added to 10mls of 80% ethanol and boiled in a water bath for two minutes. Filtrate was obtained then concentrated to crystals. Zero point five grams (0.5g) of the ethanoic extract was added to 2mls of acetic anhydride with 2mls of 0.1M sulphuric acid in test tubes and then the formation of yellow coloured layer indicated the presence of sterols according to method by Hunasagi *et al*. (2018).

3.9.6. Test for cardiac glycosides

Keller-Killiani test

Five grams of powdered samples was added to 10mls of water in test tubes the heated in water bath for 2 min and then filtered to obtain an extract. Five millilitres of each extract were added to 2mls glacial acetic acid containing one drop of 0.1% ferric chloride solution. Concentrated hydrochloric acid was then added and a blue colour appeared in the acetic layer which indicated the presence of cardiac glycosides (Emasushan & John, 2018).

3.9.7. Test for alkaloids

Mayer's Test

Two grams of dried plant powder was mixed with 40ml of 0.1M hydrochloric acid in a boiling tube then heat in water bath for 10 minutes. It was cooled then filtered. To the portion of the filtrate a few drops of mayor's reagent were added and the formation of a cream precipitate was evidence of presence of alkaloids according to method by Narayana *et al.* (2018).

3.10. Preparation of crude extracts from *Allium sativum*, *Azadirachta indica* and *Tithonia diversifolia*

Ten kilograms each of fresh *Allium sativum* bulbs, leaves of *Azadirachta indica* and *Tithonia diversifolia* were washed thoroughly under running tap water and soaked in 2% solution of sodium hypochlorite for 5min then rinsed thoroughly with sterilized distilled water (Narayana *et al*., 2018). *Allium sativum* cloves were slice cut in thin slices to fasten the drying process and all air-dried at room temperature until complete dryness was achieved. The dried *Allium sativum, Azadirachta indica* and *Tithonia diversifolia* were ground into fine powder using a laboratory blending machine. Crystal dried garlic slices were ground using mill grinder and the fine powder stored in brown airtight paper bags to prevent moisture from getting in at room temperature to await extraction. Two hundred grams of *Allium sativum, Azadirachta indica* and *Tithonia diversifolia* were weighed separately using a top loading balance (BWLC-2000-TB model) and each transferred into nine 500ml conical flasks. Five hundred millilitres of

solvents of methanol, ethanol and distilled water was added and left at room temperature in the dark for 7 days for the extraction of the bioactive compounds according to the method of Byrappa (2015). The macerated plant tissues were separated from the aqueous solution by filtering using a muslin cloth then re-filtered using Whatman filter paper No. 1. The filtrate was then sterilized by serially filtering through a 0.45 micrometre-pore-size filter and then a 0.22 micrometre-pore-size filter and the filtrate evaporated to dryness to obtain crystals by placing them in water bath at 35^{o}C overnight and then freeze-dried into fine powder for long term storage and kept at 4^{o}C in the dark (Baba and Malik, 2015). When needed, the extracts were reconstituted by dissolving in water using a stock solution of 10mls. 75% extract concentration was obtained by measuring 7.5mls of the stock solution plus 2.5mls of water, 50% extract concentration was obtained by measuring 5mls of the stock solution plus 5mls of water, 0% was achieved by measuring 10mls of sterile water and stored in the dark at 4^{o}C according to Narayana *et al*. (2018). Sterile water was used in the experiment as negative control and kept at 4^{o}C until required for use.

3.11. Effects of *Azadirachta indica, Allium sativum* and *Tithonia diversifolia* crude extracts on spore germination obtained different solvent extracts of distilled water, methanol and ethanol at 50, 75 and 100% concentrations

Antifungal activity of *Azadirachta indica, Tithonia diversifolia* and *Allium sativum* extracts was determined using the paper disc diffusion method of Esmaeili *et al*. (2013). Ten millilitres of Potato Dextrose Agar were poured per petri dish of the 144-petri dish. The fungal growth was adjusted to 0.1 of dilution potato dextrose broth. 5ml of autoclaved 0.01% Tween-20 was added to scintillation vails containing the fungus and vortex thoroughly for 1minute. Wide-bore pipette tip was then used to dilute the inoculum by transferring two separate 0.1ml aliquots of spore suspension into 2ml Eppendorf tubes containing 1.8ml of 0.01% Tween-20 after which each aliquot was enumerated. Zero point one millilitres (0.1mls) of the inoculum suspension contained approximately 10^{8} fungus/ml which were poured over the agar in the petri plates and

dispersed using sterile cotton swab. Sterile filter paper discs of 6mm diameter in dimension were soaked for 30 seconds in 10ml of plant extracts in sterile petri dishes at concentrations of 50, 75 and 100%, and immediately introduced into the centre using sterile mounting needle and forceps. Sterile water was used as negative control while synthetic fungicide (AMISTAR TOP) was used as the positive control. Inhibition zones were measured using ruler (mm) under a microscope x400. The sizes of the inhibition zones were used to quantify the extent of germination of the fungal spores after incubation at 27^{o}C for 72 hours. All the treatments were replicated four times.

3.12 Determination for the effects of methanolic extracts of *Azadirachta indica, Tithonia diversifolia* and *Allium sativum* extracts at 100% concentrations on growth component of common bean Var. GLP 1127 Mwezi moja

In vivo evaluations were conducted at Maseno University greenhouse to evaluate the fungicidal effects of methanolic extracts of *Azadirachta indica, Tithonia diversifolia* and *Allium sativum* on angular leaf spot disease of common bean. Certified seeds of GLP 1127 "Mwezi moja" were purchased from certified Simlaw seed dealer in Kisumu which were planted in plastic pots of diameter 20cm containing 6kg of solar sterilised sandy-loam soil collected in Maseno University farm with four holes drilled at the bottom to allow for effective drainage. The pots were arranged on greenhouse floor at 60cm distance from each other horizontally and 30cm from each other under uncontrolled environmental conditions and watered overhead twice a day using micro sprinklers to ensure good germination and growth. Soil was watered and four seeds of GLP 1127 Mwezi moja sowed per pot of which two were thinned immediately after the tri-foliar stage of which two well grown bean plants were during data collection. A basal dose of triple superphosphate granules fertilizer was applied during sowing at the rate of 4g/hole to all the experimental plots. Four weeks after sowing, the selection and tagging were done then all plants inoculated with 60ml suspension 2.5 x 10^{6} spore/ml of *Phaeoisariopsis griseola* which was obtained by scraping cultured plates in about 5ml of autoclaved 0.01%

tween-20 solution to liberate spores. Mycelium were removed by gravity filtering through at least 4 layers of autoclaved muslin cloth. Spore concentration was calculated with hemocytometer and adjusted to 60ml suspension 2.5 x 10^6 spore/ml and foliar sprayed using a hand-held sprayer. 7days after disease inoculation, 100% concentrated methanolic extracts of *Tithonia diversifolia*, *Azadirachta indica* and *Allium sativum* were sprayed on the common bean, some plants were sprayed with sterile water was used as negative control while others were sprayed with synthetic fungicide (AMISTAR TOP) as positive control (Obongoya *et al*., 2010). The experiment was laid out in a complete randomized design with four replicates. Data was then collected. Methanolic extract at 100% concentration was used for all the greenhouse test since it had shown the best results during the *in vitro* experiment.

3.12.1 Growth

Data on growth index was collected for quantitative indicator of plant growth. Components of growth index were plant height and stem diameter.

3.13.1.1 Plant height

Plant height (H) was measured using a meter ruler in centimetres from the soil surface at the base of the stem to the furthest point vertically. Data on plant height was collected 8 weeks from emergence.

3.13.1.2 Plant stem diameter

Plant stem diameter was measured using a Vernier calliper in centimetres. Measurement was taken at the top just below the first branch (W1) and slightly above the ground(W2). Data on plant stem diameter was collected 8 weeks from emergence.

Data on plant height and plant stem diameter were used to calculate growth index as a measure of plant growth in centimetres. The growth index was calculated as follows:

GI=(H+(W1+W2)/2)2

Where; GI = growth index

H = plant height (cm)

W1 = top stem diameter measurement (cm)

W2 = bottom stem diameter measurement (cm), according to method by Irmak *et al*. (2004).

3.13. Determination of the effect of *Azadirachta indica, Tithonia diversifolia* and *Allium sativum* extracts on yield component of common bean Var. GLP 1127 Mwezi moja

Yield component involved taking weights of common bean pods in grams and number of seeds per treatment harvested at 12^{th} week from emergence (Muthomi *et al*., 2017). The pods from common bean plants per pot were harvested and weighed using a top pan weighing balance (Intel-Lab PC-10001 Precision Balance 0.1g) and readings recorded in grams according to a method by Wahome *et al*. (2011). Whole plant fresh weight was obtained by uprooting two plants from the pot after watering the soil thoroughly. The remaining soil made into water-soil consistency then sieved to obtain parts of the roots that might have remained in the soil then data on whole plant fresh weight collected in grams using a top pan weighing balance (Muthomi *et al*., 2017). The plants were then dried in the oven at $33^{O}C$ for three days to obtain data on whole plant dry weight in grams. The data was collected and mean computed for the fresh weight and dry weight in grams (Muthomi *et al*., 2017).

3.14. Assessment of the effect of *Azadirachta indica, Tithonia diversifolia* and *Allium sativum* extracts on disease index of common bean Var. GLP 1127 Mwezi moja

Disease index was obtained by collecting data on disease incidence and disease severity. Two plants per pot were tagged for disease assessment. These were done by scoring three trifoliate leaves sampled at the bottom, middle and top of each plant at the 10^{th} week after emergence (Wahome *et al*., 2011). The total disease index was computed using score of incidence and

severity. Percentage disease indices was calculated using the formula according to Muthomi *et al*. (2017).

$$\text{Disease index (\%)} = \frac{\text{Incidence score + severity score}}{\text{Maximum incidence + maximum severity}} \times 100$$

3.14.1 Assessment of the effect of *Azadirachta indica, Tithonia diversifolia* and *Allium sativum* extracts on disease incidence on common bean

The assessment of disease incidence from all the treatments involved the counting of all diseased plants that showed symptoms of angular leaf spot infection. Data on disease incidence was collected on the 10th week from emergence and the percentage of the disease incidence calculated according to the formula by Muthomi *et al*. (2017).

Percentage disease incidence = (number of infected plants/total number of plants) x 100

Scoring was done based on a rating scale, where;

Low incidence = 1-20%

Moderate incidence = 21-49%

High incidence = 50-100%

3.14.2 Assessment of the effect of *Azadirachta indica, Tithonia diversifolia* and *Allium sativum* extracts on disease severity on common bean

Disease severity is the measure of impact of the disease on a plant and involved determination of the percentage of leaf area showing disease symptoms against the whole leaf area in a plant per treatment (Muthomi *et al*., 2017). Data on disease severity was collected on the 10th week from emergence using a standard scale of 0-5 scale according to Fikre *et al*. (2011).

To determine disease severity a scale of 0-5 was applied.

where;

0 = no disease,

1 = <20% of the leaf area infected,

2 = 21-40% of the leaf area infected,

3 = 41-60% of the leaf area infected,

4 = 61-80% of the leaf area infected

and 5 = 81-100% of the leaf area infected

3.15 Determination of Minimum Inhibitory Concentration

Minimum inhibitory concentration (MIC) helps in identifying the exact extract concentration required to inhibit fungal spore germination under controlled conditions. MIC of the extracts was determined by using broth dilution. The minimum inhibitory concentration was defined as the lowest concentration able to completely inhibit any visible microorganism growth after overnight incubation with media (Yahaya *et al*., 2017). Serial dilution of the extracts in liquid medium were prepared. One millilitre of the prepared broth was dispensed into the test tubes labelled A to D using sterile syringe and needle. A sock solution of 100mg/ml of the extracts was prepared. Then, 1.0ml of the solution was dispensed into test tube A. Subsequently, from tube A, solution was serially transferred until test tube D of 1.0ml of the solution were discarded from it. An overnight culture of the test isolate was prepared in sterile PDA agar. 1ml inoculum was transferred into each test tube from A to D. the final concentration of the extract in each of the test tubes numbered after dilution 100, 50, 25 and 2.5 mg/ml was incubated at 37^{o}C for 24hrs and examined for spore germination. To measure the MIC values, various concentrations of the stock at 12.5, 25, 50 and 100 mg/ml were assayed against the fungus.

3.16 Disposal of experimental materials

Experimental materials used in characterization and fungicidal activity tests were autoclaved to kill the fungus before being washed in detergent. Infected plant materials from the greenhouse were uprooted and burnt to eradicate and prevent spread of the fungus.

3.17 Data analysis

Data collected on morphological variation among the isolates was contrasted for any variation among the isolates. Data on phytochemical analysis of the different plants was scored for presence or absence of a particular phytochemical constituents respectively. Data on inhibition zones was collected for the selected plant extracts from different solvents, SAS Version 9.1 was used a statistical package, the data was subjected to Analysis of Variance (ANOVA) test at ($P < 0.05$) and means separated and compared using Least Significant Difference (LSD) at 5% probability level. Data on plant height and stem diameter, yield and disease index were compiled and subjected to Analysis of Variance (ANOVA) test at ($P < 0.05$) and means separated and compared using Least Significant Difference (LSD) at 5% probability level.

CHAPTER FOUR

RESULTS

4.1 Morphological profiling of *Phaeiosariopsis griseola* isolates from Sabatia, Ugenya and Bondo regions

Bondo, Sabatia and Ugenya isolates had similar cultural characteristics of flat compact colonies, white at the start then became black homogenously and a grey underside. Microscopically, the pathogen isolated from Sabatia sub-county was observed to be aseptate and hyphae with erect, simple and thick walled conidiophore bearing a head and black in colour while its spores were elliptical in shape, double-walled, unicellular, grey in colour and non-attached (Plate 4.1c and 4.1d, and Table 4.1). Ugenya isolate was aseptate and several had fragmented hyphae with thin walled conidiophores bearing heads split into several loose conidial columns and black in colour while spores were found to be circular, unicellular, double-walled, grey and no attachment (Plates 4.1e and 4.1f) Table 4.1). *Phaeiosariopsis griseola* isolate from Bondo sub-county was observed to be septate and hyphae with erect, simple and thick-walled conidiophore bearing a head and black in colour while spores appeared lemon shaped, grey, unicellular, double walled and non-attached (Plates 4.1a and 4.1b) Table 4.1.

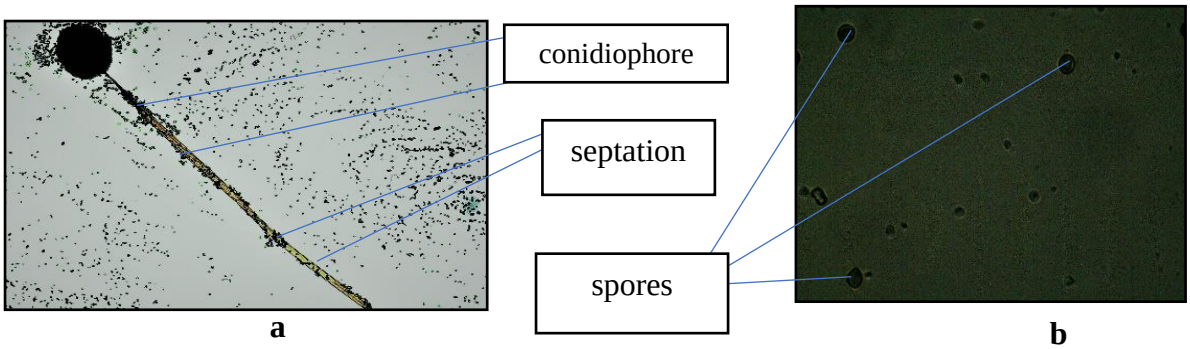
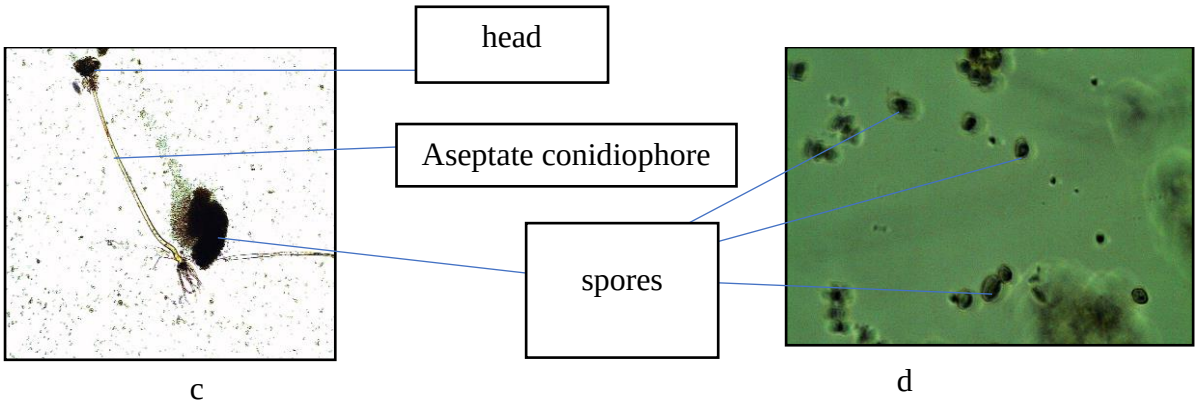


Plate 4.1: Bondo isolate hyphae (a) and Bondo isolate spores (b) mg. x400 showing morphological characteristics.



Pllate 4.1: Sabatia isolate hyphae (c) and Sabatia isolate spores (d) mg. x400

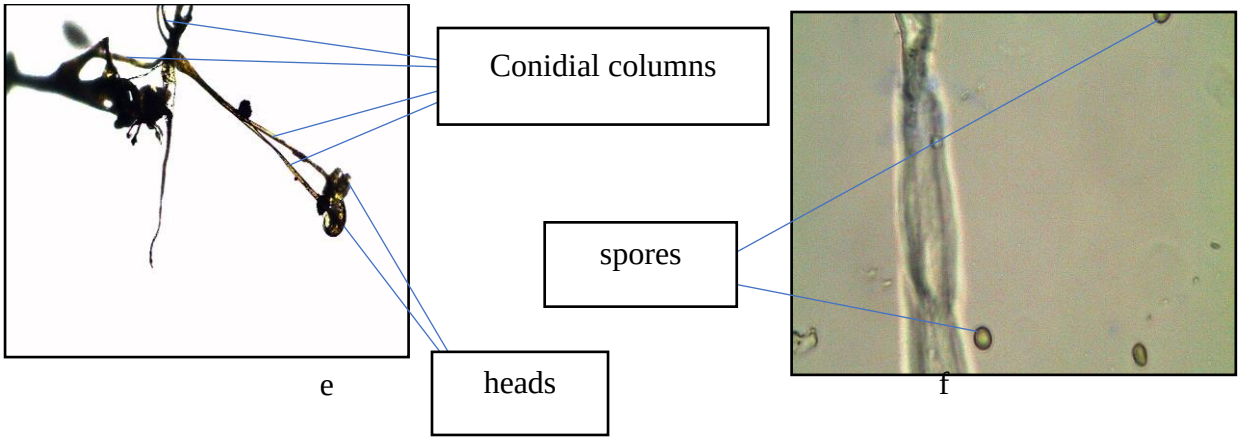


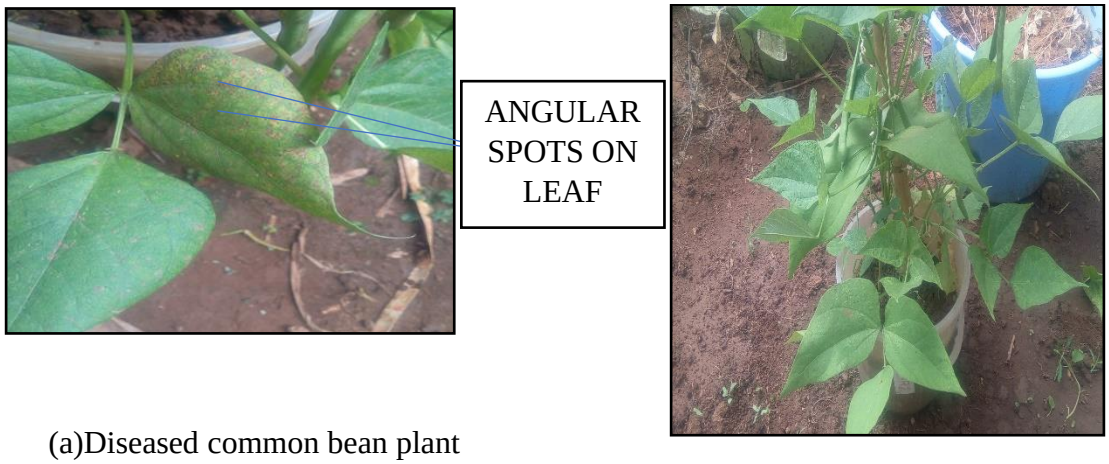
Plate 4.1: Ugenya isolate hyphae (e) and Ugenya isolate spores (f) mg. x400

Table 4.1. Morphological characterisation of isolates of *Phaeiosariopsis griseola*

Hyphae characteristics	**Sabatia isolate**	**Ugenya isolate**	**Bondo isolate**
Septation	Aseptate	Septate	Septate
Colour	Black	Black	Black
Spore characteristics			
Spore shape	Elliptical	circular	lemon-shaped
Number of cells	Unicellular	Unicellular	Unicellular
Spore colour	Grey	Grey	Grey
Wall characteristics	double	Double	Double

4.2 Test of pathogenicity of the isolates

Phaeiosariopsis griseola was isolated from Sabatia, Bondo and Ugenya common bean plants and its pathogenicity was confirmed by verification of Koch's postulations. Common bean plants inoculated with Sabatia, Bondo and Ugenya isolates showed typical angular leaf spot symptoms of small, angular, dark brown and numerous spots on the leaves, the interaction was considered pathogenic (Plate 4.4 (a)). At first, the lesions were grey, later turn brown and attain an angular shape because of limitation by veins. On all lesions, dark stroma appeared in abundance. The uninoculated common bean plants showed no symptoms of angular leaf spot disease hence served as control (Plate 4.4 (b)).



(a)Diseased common bean plant

(b)Healthy common bean plant

Plate 4.4. Symptoms of angular leaf spot disease on (a) diseased common bean plant and (b)a healthy plant. (Courtesy of Simon Meso).

4.3. Phytochemical screening of *Azadirachta indica*, *Tithonia diversifolia* and *Allium sativum*

4.3.1 Test for tannins

The test for tannins in *Azadirachta indica, Tithonia diversifolia* and *Allium sativum* showed a change in colour to brownish green of black which indicated a positive test (Table 4.2).

4.3.2 Test for saponins

There was formation of emulsion which indicated the presence of saponins in *Azadirachta indica* and *Allium sativum* unlike for *Tithonia diversifolia* where no formation of emulsion was observed which indicated the absence of saponins (Table 4.2).

4.3.3 Test for flavonoids

There was no colour change which indicated a negative test for flavonoids in *Azadirachta indica* unlike for *Tithonia diversifolia* and *Allium sativum* showed a colour change of yellow colouration which indicated a positive test (Table 4.2).

4.3.4 Test for terpenoids

There was the formation of an interface of reddish-brown colouration that indicated the presence of terpenoids in *Azadirachta indica, Tithonia diversifolia* and *Allium sativum* (Table 4.2.).

4.3.5 Test for sterols

There was a colour change from violet to blue which indicated a positive test for sterols in *Azadirachta indica* and *Allium sativum* while the colour remained violet for *Tithonia diversifolia* which indicated the absence of sterols (Table 4.2).

4.3.6 Test for cardiac glycosides

A violet ring appeared below the acetic acid layer and a greenish ring also formed gradually throughout the thin layer. This indicated the presence of cardiac glycosides in *Azadirachta indica, Tithonia diversifolia* and *Allium sativum* (Table 4.2).

4.3.7 Test for alkaloids

The formation of a slightly turbid of heavy precipitate indicated the presence of alkaloids in *Azadirachta indica* and *Allium sativum* unlike *Tithonia diversifolia* where no formation of turbidity was observed indicated the absence of alkaloids (Table 4.2).

Table 4.2. Qualitative phytochemical screening of *Azadirachta indica*, *Tithonia diversifolia*, and *Allium sativum*

	Plant material		
Phytochemical	*Azadirachta indica*	*Tithonia diversifolia*	*Allium sativum*
Tannins	+	+	+
Saponins	+	_	+
Flavonoids	_	+	+
Terpenoids	+	+	+
Sterols	+	_	+
Cardiac glycosides	+	+	+
Alkaloids	+	-	+

Key: **+** present; **-** absent

4.4 Effect of *Azadirachta indica, Allium sativum* and *Tithonia diversifolia* crude extracts on spore germination obtained using different solvent extracts of distilled water, methanol and ethanol of concentration 50%, 75% and 100 %

The findings showed that all the treatments differed significantly compared to the controls on spore germination *in vitro* (Table 4.3.). However, synthetic fungicide (AMISTAR TOP) showed a higher performance than all the plant extracts at 50%, 75% and 100% concentrations. All the plant extracts at different concentrations differed significantly although, *Allium sativum* methanolic extract at all the concentrations showed a higher performance followed by methanolic *Azadirachta indica* extract at all concentrations and lastly *Tithonia diversifolia* at all concentrations. Both methanolic, ethanoic and water extracts of at 50%, 75% and 100% concentrations also showed higher performance than sterile water (control) (Table 4.3). The Sterile water (control) had no effect on fungus spore germination. The activity of all the plant extracts at different concentrations were significantly different. However, at 100% extracts concentrations for all the plant extracts showed a higher activity on spore germination followed with 75% extracts concentrations while 50% extracts concentrations had the least fungicidal activity. Among the solvent extracts at different concentrations methanolic extracts of all the plants showed a higher activity than ethanoic extract. Water extracts had the least activity (Table 4.3).

Table 4.3. Effect of *Azadirachta indica, Allium sativum* and *Tithonia diversifolia* crude extracts on spore germination using different solvent extracts of distilled water, methanol and ethanol at different extract concentration of 50%, 75% and 100%, sterile water and synthetic fungicide (AMISTAR TOP)

Plant material	Concentrations (%)	Solvent extracts		
		Methanol	Ethanol	Distilled water
Allium sativum	Fungicide	10.45±0.18a	10.80±0.04a	9.35±0.09a
	100	8.03±0.13b	7.36±0.16b	7.09±0.50b
	75	7.35±0.10c	6.95±0.05c	6.65±0.26c
	50	6.78±0.06d	6.45±0.06d	6.36±0.15d
	Control	6.00±0.00e	6.00±0.00e	6.00±0.00e
	LSD	**0.29**	**0.22**	**0.17**
Azadirachta indica	Fungicide	10.45±0.18a	10.80±0.04a	9.35±0.02a
	100	7.98±0.18b	7.51±0.07b	6.94±0.12b
	75	7.14±0.08c	7.00±0.06c	6.65±0.08c
	50	6.70±0.07d	6.50±0.05d	6.35±0.08d
	Control	6.00±0.00e	6.00±0.00e	6.00±0.00e
	LSD	**0.32**	**0.13**	**0.18**
Tithonia diversifolia	Fungicide	10.45±0.18a	10.80±0.04a	9.35±0.02a
	100	7.30±0.15b	7.23±0.13b	7.06±0.13b
	75	6.94±0.09c	6.69±0.08c	6.65±0.06c
	50	6.51±0.07d	6.40±0.05d	6.41±0.04d
	Control	6.00±0.00e	6.00±0.00e	6.00±0.00e
	LSD	**0.30**	**0.19**	**0.18**

Legend: Means followed by different letters down the columns differ significantly at P=0.05. ± standard error. Each value is an average of four replicates.

4.5 Effects of *Azadirachta indica, Allium sativum* and *Tithonia diversifolia* methanolic crude extracts at 100% concentrations on the growth and yield components of common bean Var. GLP 1127 Mwezi moja infected with *Phaeoisariopsis griseola*

4.5.1 Effects of *Azadirachta indica, Allium sativum* and *Tithonia diversifolia* methanolic crude extracts at 100% concentrations on the growth of common bean Var. GLP 1127 Mwezi moja infected with *Phaeoisariopsis griseola*

Synthetic fungicide (AMISTAR TOP), *Tithonia diversifolia*, and *Azadirachta indica* did not differ significantly but the synthetic fungicide and *Tithonia diversifolia* differed significantly with *Allium sativum* plant extract. *Allium sativum* and *Azadirachta indica* did not differ significantly compared to the negative control (Table 4.4). Synthetic fungicide (AMISTAR

TOP) and the plant extracts all showed significant effects on growth index of the common bean plants. However, Synthetic fungicide (AMISTAR TOP) showed a higher performance than the other treatments.

Table 4.4. Effects of *Azadirachta indica, Allium sativum* and *Tithonia diversifolia* methanolic crude extracts at 100% concentrations on growth components of common bean Var. GLP 1127 Mwezi moja infected with *Phaeoisariopsis griseola* isolate

Treatments	**Growth index (cm)**
Fungicide (AMISTAR TOP)	39.88±0.45a
Tithonia diversifolia	39.83±1.03a
Azadirachta indica	37.13±0.86ab
Allium sativum	34.18±1.49b
Control (sterile water)	28.80±1.90c
LSD	3.78

Legend: Means followed by different letters down the column are statistically different at P=0.05**.** ± standard error.

4.5.2 Effects of *Azadirachta indica, Allium sativum* and *Tithonia diversifolia* methanolic crude extracts at 100% concentrations on the yield components of common bean Var. GLP 1127 Mwezi moja infected with *Phaeoisariopsis griseola*

4.5.2.1 Effects of *Azadirachta indica, Allium sativum* and *Tithonia diversifolia* methanolic crude extracts at 100% concentrations on pod weight of common bean Var. GLP 1127 Mwezi moja infected with *Phaeoisariopsis griseola*

The effects of *Tithonia diversifolia* did not significantly differ with synthetic fungicide which differed significantly with *Azadirachta indica*. The effect of *Azadirachta indica* did not differ significantly with that of synthetic fungicide compared to the control (Table 4.5). *Allium sativum* methanolic extract showed a higher performance than all the treatments on pod weights

and differed significantly with *Azadirachta indica* and synthetic fungicide but did not differ significantly with *Tithonia diversifolia*.

Table 4.5. Effects of *Azadirachta indica, Allium sativum* and *Tithonia diversifolia* methanolic crude extracts at 100% concentrations on pod weight of common bean Var. GLP 1127 Mwezi moja infected with *Phaeoisariopsis griseola*

Plant extracts	**Pod weight (g)**
Allium sativum	19.10±0.97a
Azadirachta indica	12.85±0.66c
Tithonia diversifolia	16.53±1.46ab
Fungicide (AMISTAR TOP)	14.95±1.24bc
Control (sterile water)	9.05±0.82d
LSD	3.22

Legend: Means followed by different letters down the column are statistically different at P=0.05**.** ± standard error.

4.5.2.2 Effects of *Azadirachta indica, Allium sativum* and *Tithonia diversifolia* methanolic crude extracts at 100% concentrations on number of seeds of common bean Var. GLP 1127 Mwezi moja infected with *Phaeoisariopsis griseola*

Allium sativum and synthetic fungicide did not differ significantly but both differed significantly with *Azadirachta indica* and *Tithonia diversifoila* which did not differ significantly compared to the control (Table 4.6). Synthetic fungicide showed the highest effect in terms of numbers of seeds among all the treatments. *Allium sativum* showed the highest effect of the plant extracts.

Table 4.6. Effects of *Azadirachta indica, Allium sativum* and *Tithonia diversifolia* methanolic crude extracts at 100% concentrations on number of seeds of common bean Var. GLP 1127 Mwezi moja infected with *Phaeoisariopsis griseola*

Plant extract	Number of seeds
Allium sativum	36.50±1.85a
Azadirachta indica	27.75±1.75b
Tithonia diversifolia	26.75±2.69b
Fungicide (AMISTAR TOP)	39.25±1.11a
Control (sterile water)	13.75±1.75c
LSD	5.72

Legend: Means followed by different letters down the column are statistically different at P=0.05**.** ± standard error.

4.5.2.3 Effects of *Azadirachta indica, Allium sativum* and *Tithonia diversifolia* methanolic crude extracts at 100% concentrations on whole plant fresh weight of common bean Var. GLP 1127 Mwezi moja infected with *Phaeoisariopsis griseola*

Synthetic fungicides and *Allium sativum* showed no significant difference although both differed significantly with *Azadirachta indica* and *Tithonia diversifolia*. Effects of *Allium sativum* and *Azadirachta indica* did not differ significantly. Similarly, *Azadirachta indica* and *Tithonia diversifolia* did not differed significantly compared to sterile water (control) (Table 4.7). Synthetic fungicide showed a higher performance than all the treatments. Among the plant extracts *Allium sativum* showed the higher effect than the other plant extracts.

Table 4.7. Effects of *Azadirachta indica, Allium sativum* and *Tithonia diversifolia* metanolic crude extracts at 100% concentrations on whole plant fresh weight of common bean Var. GLP 1127 Mwezi moja infected with *Phaeoisariopsis griseola*

Plant extract	Whole plant Fresh weight(g) (shoot plus roots)
Azadirachta indica	60.90±3.15bc
Allium sativum	66.83±5.91ab
Tithonia diversifolia	51.65±3.52c
Fungicide (AMISTAR TOP)	76.95±3.22a
Control (sterile water)	27.78±3.01d
LSD	11.81

Legend: Means followed by different letters down the column are statistically different at P=0.05**.** ± standard error.

4.5.2.4 Effects of *Azadirachta indica, Allium sativum* and *Tithonia diversifolia* methanolic crude extracts at 100% concentrations on whole plant dry weight of common bean Var. GLP 1127 Mwezi moja infected with *Phaeoisariopsis griseola*

Synthetic fungicides and *Allium sativum* did not differ significantly although both differed significantly with *Azadirachta indica* and *Tithonia diversifolia*. Effects of *Allium sativum* and *Azadirachta indica* did not differ significantly. Similarly, *Azadirachta indica* and *Tithonia diversifolia* did not differed significantly compared to sterile water(control) (Table 4.8). However, synthetic fungicide performed higher than all the treatments. Among the plant extracts *Allium sativum* showed the higher effect than the other plant extracts.

Table 4.8. Effects of *Azadirachta indica, Allium sativum* and *Tithonia diversifolia* methanolic crude extracts at 100% concentrations on whole plant dry weight of common bean Var. GLP 1127 Mwezi moja infected with *Phaeoisariopsis griseola*

Plant extract	**Whole plant Dry weight(g) (shoot plus roots)**
Azadirachta indica	13.65±1.30bc
Allium sativum	18.00±3.60ab
Tithonia diversifolia	11.65±1.20c
Fungicide (AMISTAR TOP)	23.90±2.11a
Control (sterile water)	11.05±0.90c
LSD	6.24

Legend: Means followed by different letters down the column are statistically different at P=0.05**.** ± standard error.

4.6 Effect of *Azadirachta indica, Allium sativum* and *Tithonia diversifolia* methanolic crude extracts at 100% concentrations on disease index of angular leafspot disease in common bean Var. GLP 1127 Mwezi moja

All the treatments differed significantly with the control. However, synthetic fungicide (AMISTAR TOP) performed higher than all the treatments. All the plant extracts also differed significantly with the control although, among the plant extracts *Allium sativum* showed higher performance than *Tithonia diversifolia* and *Azadirachta indica* on disease index compared the control (P=0.05) (Table 4.9).

Table 4.9. Effect of *Azadirachta indica*, *Allium sativum* and *Tithonia diversifolia* methanolic crude extracts at 100% concentrations on disease index of *Phaeiosariopsis griseola* on common bean Var. GLP 1127 Mwezi moja

Plant extract	**Disease index**
Azadirachta indica	40.68±0.67c
Allium sativum	27.56±0.53d
Tithonia diversifolia	55.32±1.32b
Fungicide (AMISTAR TOP)	18.37±2.4e
Control (sterile water)	83.65±1.52a
LSD	4.44

Legend: Means followed by different letter down the column are statistically different at P=0.05 by Duncan test**.** ± standard error.

CHAPTER FIVE

DISCUSSIONS

5.1. Morphological characterisation of isolates of *Phaeiosariopsis griseola* of common bean Var. GLP 1127 Mwezi moja

Cultural characterisation of *P. griseola* revealed similarities among the different isolates with all of the isolates showing compact colonies with a grey underside. Significant variations among Bondo, Sabatia and Ugenya isolates were observed in terms of hyphae and spore microscopically. From the findings of this study the conidial size, septation and number of conidiophores among the isolates of *Phaeiosariopsis griseola* were found to vary which is in agreement with the findings of Crous *et al*. (2006). The presence of different strains of *Phaeiosariopsis griseola* in a given location is an important area which many studies have tended to overlook. If different strains are present but not identified, confusing and conflicting results can be obtained while dealing with other aspects of the fungus especially during screening and breeding for resistance. These morphological variations could have been associated with mutation, recombination and migration. Also, specialisation in host–pathogen interactions, control measures or more general environmental constraints may singly or interact in combination to give raise to new pathotypes leading to high levels of diversity in the pathogen.

5.2 Pathogenicity test of the *Phaeiosariopsis griseola* isolates

Considering the importance of correct identification of plant pathogens, *Phaeiosariopsis griseola* causing angular leaf spot disease in common bean Var. GLP 1127 Mwezi moja was identified using pathogenicity approach. There was a positive pathogenicity outcome. The findings concurred with studies by Ddamulira *et al*. (2014) and Sartorato (2002) who obtained similar results when *Phaeiosariopsis griseola* was artificially inoculated with common bean. These results demonstrated the fact that all the fungi isolates investigated for pathogenicity were indeed pathogenic.

5.3. Phytochemical analysis of *Azadirachta indica, Allium sativum* and *Tithonia diversifolia* plant parts

The present work which was undertaken to screen for phytochemical compounds for potential fungicidal activity against *Phaeiosariopsis griseola* that causes angular leaf spot diseases in common bean, confirmed the presence of several phytochemical constituents with fungicidal ability in *Azadirachta indica, Allium sativum* and *Tithonia diversifolia* (Table 4.2). The results are in agreement with Ramadass (2018) that reported that plants have several antifungal compounds. Most phytochemicals play an important role in protection against fungal pathogens by affecting pathogens' physiology, morphology and ultrastructure or indirectly by promoting plant systemic resistance (Sales *et al*., 2016). A plant that exhibits antimicrobial activities should be tested against appropriate microbes to confirm the activity and to ascertain the parameters responsible for the activity Shabana *et al*. (2017) and this led to the results obtained from this research work on phytochemical screening of *Azadirachta indica, Tithonia diversifolia,* and *Allium sativum* plant parts. The phytochemical investigation of *Allium sativum* bulbs revealed the presence of alkaloids, flavonoids, saponin, tannins and cardiac glycoside. The findings are in agreement with the work done by Idowu *et al*. (2008); Hunasagi *et al*. (2018) which reported that these classes of compounds especially alkaloids, saponins, tannins and flavonoids are known to have fungicidal activity against several pathogens. Thus, the presence of these phytochemicals confirmed the fungicidal activities of extract of *Allium sativum* bulb. Phytochemical screening of the leaves extract of *T. diversifolia* revealed that among the substances investigated, presence of phenolic compounds was detected (total phenols, tannins and flavonoids) while alkaloids, sterols and saponins were not detected (Table 4.3). The presence of some of these secondary metabolites suggest that the plant might be of fungicidal importance which was in agreement with results of Rasaga *et al*. (2014) differing

only in the presence of sterols which may be attributed to the part used to obtain the extract in the study. Rasaga *et al*. (2014) used flowers of *T. diversifolia*. The difference in the phytochemical composition from these two studies could be due to variable distribution of phytochemicals compounds in different parts of *Tithonia diversifolia* plant. Similar results were also reported with the earlier studies of Ogundare (2007) and Rejeki & Addy, 2017) on the availability of phytochemical compounds within most parts of *Tithonia diversifolia*. From the test of *Azadirachta indica*, the following biologically active compounds were tested positive; alkaloids, saponins, tannins, terpenoids, cardiac glycosides and sterols which is partially in agreement with the findings of Ahmed (2008) on the absence of flavonoids. These class of compounds especially terpenoids, alkaloids, saponins and tannins are known to have antimicrobial activity against several pathogens (Rejeki & Addy, 2017). The exploitation of plant products for the management of plant diseases have achieved greater significance in recent times due to its readily available nature, antimicrobial activity, easy biodegradability, non-phytotoxicity, besides inducing resistance in host.

5.4. Effect of *Azadirachta indica, Allium sativum* and *Tithonia diversifolia* crude extracts on spore germination obtained using different solvent extracts of distilled water, methanol and ethanol

The findings showed the inhibition of spore germination *in vitro* with all tested extracts with the most effective being methanolic *Allium sativum* extract at 100% concentration. *Allium sativum* is a spice with global recognition. In this study, *Allium sativum* had inhibited spore germination when tested. The inhibitive effect was proportional to the concentration for all the plant extracts of *Allium sativum, Azadirachta indica* and *Tithonia diversifolia*; at higher extract concentrations there was higher inhibition activity by the plant extracts. These effects are in accordance with the results of Singh *et al*. (2014) who reported that garlic extract had effective spore germination inhibition on *Alternaria dauci*. The fungicidal action of *Allium sativum* is due to the compound allicin. It has strong antimicrobial and antifungal activities (Keerio *et al*.,

2017). Thus, inhibition of fungi observed in this study may be related to allicin or ajoene which curbs the performance of some enzymes that are important to fungi. The results clearly indicate that the *Allium sativum* methanolic extract showed the highest inhibitory activity. The water extracts of *Allium sativum* showed least fungicidal activity than the ethanolic extract against the test organism, which is in agreement with earlier report by Abdulaziz *et al*. (2018) which reported that methanol had a higher extraction ability than ethanol and water. *Allium sativum* has been reported to have fungicidal activity on *Puccinia tritina* a wheat fungus that causes wheat leaf rust (Shabana *et al*., 2017). Similar results were reported by Keerio *et al*. (2017) with *Allium sativum* being effective in controlling *Fusarium oxysporum* fungus. The fungicidal activity of *Azadirachta indica* leaves extract against fungal plates showed considerably spore germination inhibition activity similar findings were also presented by Ravishankar *et al*. (2018) that alcoholic extracts of *Azadirachta indica* had high inhibitory effect on fungus; the fungus targeted were *Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger* and *Aspergillus terreus*. The leaves extract of *Azadirachta indica* and *Tithonia diversifolia* plant has been previously reported to show antifungal activity Parekh & Chanda (2007). Effectiveness of *Azadirachta indica* leaves extract in this experiment also agree with the work of Pankaj *et al*. (2011) which reported its antifungal activity against *Trichophyton mentagrophytes*. The results also in agree with the findings of Keerio *et al*. (2017) in which it was found to have fungicidal activity against *Fusarium oxysporum* fungus. The results also conform to Ezeonu *et al*. (2018) who reported that 5% aqueous leaf extract of neem was shown to cause inhibition in growth of six tested fungal pathogens (*Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Aspergillus terreus, Candida albicans* and *Microsporum gypseum*) and further confirmed that aqueous neem extracts inhibited *A. niger* more than *C. albicans*, while alcohol neem extract inhibited *C. albicans* better than *A. niger*. The highest extraction potential effect was observed with methanol solvent while water solvent had the

least effect. Similar results were reported by Sultana *et al*. (2009) that aqueous methanol was found to be more effective in recovering highest amounts of phenolic compounds from rice bran and *Moringa oleifera* leaves than ethanol and water. Extracts obtained using methanol as solvent had higher activity followed by ethanol and then water. This shows that the extraction yield increases with increasing polarity of the solvent used in extraction. The results of this study are in agreement with the findings of Quy *et al*. (2014). From the experiment done, about three quarter of the results showed that as the concentrations of the plant extracts increases the inhibition effectiveness of the extracts also increased which is partly in agreement with the findings of Effiong *et al*. (2016). These results were also in agreement with the findings of Onaran *et al*. (2016) that concluded; the plant extracts showed a different level of antifungal activities in a dose depend manner.

5.5 Effects of *Azadirachta indica, Allium sativum* and *Tithonia diversifolia* crude extracts on the growth and yield components of common bean Var. GLP 1127 Mwezi moja infected with *Phaeoisariopsis griseola*

From this study there was significant increase in the growth index in *Azadirachta indica, Allium sativum* and *Tithonia diversifolia* extract treatments in comparison to the control. All measured parameters displayed significant differences from their respective controls. *Azadirachta indica, Allium sativum* and *Tithonia diversifolia* extracts showed a promotive effect on plant height and stem diameter with increasing time compared to control ones. The promotive effect could be due to triterpene which acted by delaying the transformation of ammonium nitrogen into nitrate nitrogen as reported by Al hazmi (2013). Growth stimulating effect of some medicinal plant extracts *P. pinatta, A. marmelos, A. indica, B. campestris, P. nigrum, E. tirucalli* have been observed (Pattnaik *et al*., 2012). Okunlola and Thomas (2013) also reported *Azadirachta indica* extract had effect on the growth of jute under sole and mixed cropping system. The growth stimulating effect was not exclusively due to its adverse effect on pathogen or by an increase in nutrient uptake. However, substances with hormone like properties can stimulate of

effect biomass allocation in plants. In addition to hormones, medicinal plant extracts contain saponins and polyphenols which could be the active compounds causing the effect on growth (Anderson, 2010).

The current study showed a significant increase in the yield components after application of *Azadirachta indica, Allium sativum* and *Tithonia diversifolia* extract treatments in comparison to the control. All measured parameters displayed significant differences from their respective controls. *Azadirachta indica, Allium sativum* and *Tithonia diversifolia* extracts showed a promotive effect on pod weight, number of seeds, fresh and dry weights with increasing time compared to control ones. The promotive effect could be due to triterpene which acted by delaying the transformation of ammonium nitrogen into nitrate nitrogen as reported by Al hazmi (2013). Yield stimulating effect of some medicinal plant extracts *P. pinatta, A. marmelos, A. indica, B. campestris, P. nigrum, E. tirucalli* have been observed (Pattnaik *et al*., 2012). Okunlola and Thomas (2013) also reported *Azadirachta indica* extract had effect on the yield of jute under sole and mixed cropping system. The yield stimulating effect was not exclusively due to its adverse effect on pathogen or by an increase in nutrient uptake. However, substances with hormone like properties can stimulate of effect biomass allocation in plants. In addition to hormones, medicinal plant extracts contain saponins and polyphenols which could be the active compounds causing the effect on yield (Anderson, 2010).

5.6. Effect of *Azadirachta indica, Allium sativum* and *Tithonia diversifolia* crude extracts on disease index of angular leaf spot disease in common bean Var. GLP 1127 Mwezi moja

In this study, AMISTAR TOP (synthetic fungicide) revealed highest activity on reducing disease index. Similarly, among the plant extracts *Allium sativum* was the most effective of the plant extracts which is in agreement with the findings of Nashwa and Abo-Elyousr (2012) who reported that most effective treatments with plant extracts were *A. sativum* at 1% and 5% concentration, followed by *D. stramonium* at 1% and 5% concentration against early blight

disease caused by *Alternaria solani* similar findings were also echoed by Yanar *et al*. (2011). This study showed that the crude extracts of *Azadirachta indica, Allium sativum* and *Tithonia diversifolia* exhibit fungicidal effect on *Phaeoisariopsis griseola*. The results confirmed that *Allium sativum* was observed have most of the phytochemicals (glycosides and saponins) compared to *Azadirachta indica,* and *Tithonia diversifolia* which have antifungal activity which is in agreement with Shabana *et al*. (2017); Ezeonu *et al*. (2018) which reported that plants with most phenolic compounds have high efficiency against plant pathogens because of their antimicrobial activity. *Azadirachta indica* was reported in this study to substantially reduce disease index compared to *Tithonia diversifolia* , this conform to findings of Enikuomehin (2005) which reported that leaf extracts of *Azadirachta indica* substantially reduced the number of infected leaves and number of lesions on foliage, and curtail disease development, which in turn, protected flowers and capsules from infection. Obongoya *et al*. (2010) reported that *Azadirachta indica* had the potential of reducing disease incidence of *Fusaruim oxysporum* of common bean by about 17% findings echoed by Ezeonu *et al*. (2018) that neem had significantly($P<0.05$) inhibited the growth of the fungal organisms against Cocoyam rot. The results from this study revealed that all the plant extracts had shown a positive effect on reducing *Phaeoisariopsis griseola* pathogen infection. Therefore, these extracts could be useful in the control of *Phaeoisariopsis griseola* fungus of common bean. The results indicated that various crude extracts had fungicidal activity. This may be attributed to the secondary metabolites of the extracts, including terpenoids, flavonoids and other phenols. These metabolites work antagonistically and as a result the pathogen can never develop resistance (Njue *at al*., 2014). This further strengthens this study on *Phaeoisariopsis griseola* control of common bean by *Azadirachta indica, Allium sativum* and *Tithonia diversifolia* extract. A study was undertaken to evaluate the effectiveness of 33 plant extracts against leaf spot of ground nut. All treatments including *Azadirachta indica, Allium sativum* and *Tithonia*

diversifolia gave considerable reduction in disease incidence and severity (Hussain *et al*., 2013). Qualitative screening of phytochemical compounds of *Azadirachta indica, Allium sativum* and *Tithonia diversifolia* showed the presence of phenols, flavonoids, tannins, alkaloids and saponins (Table 4.2). These class of compounds independently or in combination may be responsible for the broad range of fungicidal properties of *Azadirachta indica, Allium sativum* and *Tithonia diversifolia*. These plants contain at least 35 biologically active phenolic compounds which are present predominantly in the seeds, leaves and other parts of the plant Mondall *et al*. (2009); Nahak and Sahu (2010) are the most active fungicidal ingredients for effective control of angular leaf spot disease of common bean. Interestingly the important issue that must be noticed in the present study is the effectiveness of synthetic fungicide (AMISTAR TOP) which was the positive control for the study appeared to be most effective in terms of disease index reduction of angular spot disease in common bean under greenhouse and *in vitro* this could be attributed to the mode of action of its active components: Azoxystrobin and Difenoconazole**,** Azoxystrobin inhibits spore germination at early stages of fungal development thereby conferring excellent protection against invasion by fungal pathogens. It is also active against post germination stages of the life cycle in a broad range of fungal species which confers anti-sporulant activity against a wide range of pathogens. Difenoconazole's mode of action permits protective and curative use. It is taken up by the plant and acts on the fungal pathogen during penetration and haustoria formation. It stops development of the fungi by interfering with the biosynthesis of sterols in cell membranes, similar findings were also reported by Ezeonu *et al*. (2018) where ketoconazole which was used as the positive control showed 100% inhibition against the growth of the fungi *A. oryzae* and *A. niger*. However, easy availability of plant species coupled with less phytotoxicity and environmental hazards make plant extracts a potential alternative (Cherkupally *et al*., 2017). Most plants contain substances that can be used for anti-microbial purpose of which are precursors for the synthesis of useful

botanicals. Crude extracts of plants have been used as antimicrobial (Shabana *et al*., 2017). The antimicrobial value of these plants lies in bioactive phytochemical constituents that produce definite physiological action on the phytopathogens.

CHAPTER SIX

CONCLUSION, RECOMMENDATIONS AND SUGGESTIONS FOR FURTHER RESEARCH

6.1 Conclusion

The study has revealed the existence of morphological variations of *Phaeiosariopsis griseola* among the isolates from Bondo, Sabatia and Ugenya. These morphological variations may be attributed to a number of factors ranging from environmental, host type among other factors.

The phytochemical screening confirmed that *Azadirachta indica*, *Allium sativum* and *Tithonia diversifolia* plants are rich in phytochemicals. The findings confirmed the presence of phytochemicals like alkaloids, saponins, tannins, cardiac glycosides, terpenoids, flavonoids and sterols in nearly all the plants.

These plants have confirmed fungicidal activity on spore germination of *Phaeiosariopsis griseola* fungus but there was some degree of variation in their fungicidal activities. This study showed that all the plants contained most of the active phytochemical compounds which justify their fungicidal property and extract effectiveness increased with the concentration. Methanol solvent was confirmed to have the highest extraction potential followed with ethanol and lastly sterile water. This finding confirms that the more polar a solvent is the more its extraction potential and vice versa.

All the various types of plant extracts were found to be effective against *Phaeiosariopsis griseola* fungi-induced in common bean Var. GLP 1127 Mwezi moja examined. Therefore, there is possibility that *Azadirachta indica, Allium sativum* and *Tithonia diversifolia* extracts can control *Phaeiosariopsis griseola* of common bean. *Allium sativum* methanolic extracts at high concentrations should be used in controlling and management of angular leaf spot disease of common bean.

There is need to harness the potential of these plant extracts which are eco-friendly and biologically degradable to control *Phaeiosariopsis griseola* pathogen as it would help ameliorate the cost and negative effects of continuous use of synthetic fungicides. Considering the high fungicidal activity of *Azadirachta indica, Allium sativum* and *Tithonia diversifolia* extracts under the test conditions, the extracts may be strong candidates for future field tests. The study revealed significant findings that would be beneficial for the common bean producers.

6.2 Recommendation

Effective pathogen management is anchored on pathogen identification and characterization, it is therefore recommended that for proper disease control appropriate tools such as pathogenicity tests should be done for accurate pathogen identification.

The leaves extract of *Azadirachta indica and Tithonia diversifolia* and bulbs of *Allium sativum* had fungicidal activity against *Phaeoisariopsis griseola* and therefore should be used to inhibit its spore germination and growth.

The *Azadirachta indica, Allium sativum* and *Tithonia diversifolia* plants should be used in management and control of angular leaf spot as they were effective in disease management.

Methanolic extract should be encouraged in control of angular leaf spot disease as it was more effective in inhibiting the growth of *Phaeoisariopsis griseola*. Therefore, methanol as extraction solvent should be encouraged for plant extraction because of its high extraction potential compared to ethanol and sterile water.

Allium sativum should be used in the control of angular leaf spot disease of common bean as it was the most effective than *Azadirachta indica and Tithonia diversifolia* in control of disease incidence and severity.

Higher concentrations of about 50% to 100% of the extract should be used when controlling the disease to achieve better results.

6. 3 Suggestions for further research

1. It was not possible to obtain good distinct differences among the isolates of *Phaeiosariopsis griseola* from the different regions it would be prudent for future studies to conduct molecular profiling to give clear variations.
2. Isolates characterized were obtained in Bondo, Sabatia and Ugenya sub counties and its recommended that other isolates should be obtained from other regions of the country to determine whether there are existing variations.
3. Extensive research still needs to be done on phytochemicals of this plant for the development of cost-effective drugs for the future. More so, since many of the existing synthetic drugs cause various side effects, drug development using plant-based compounds could be useful in meeting this demand for newer drugs with minimal side effects.
4. Fungicidal activity of *Azadirachta indica, Allium sativum* and *Tithonia diversifolia* was determined using crude extract of the plants and there is need for more studies to be carried out to determine the individual compounds that have fungicidal activity.
5. Parts of the plants used in this study were leaves of *Azadirachta indica and Tithonia diversifolia* and bulbs of *Allium sativum*; therefore, more studies should be carried out using flowers, roots and bark to determine their fungicidal activity.
6. Disease incidence and severity reduction using the extracts were carried out in the greenhouse under controlled environment and therefore we recommend that similar studies be carried out under natural environment to determine their effectiveness.

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APPENDICES

Appendix 1: PDA media preparation

During isolation of fungi, an artificial growth medium Potato Dextrose Agar (PDA) used was prepared according to the manufacturer's specifications by ingredients such as potatoes infusion (200g), dextrose (2g), Agar (15g) and distilled water to make the volume 500ml which were sterilized in an electric operated autoclave at 15 PSI for 15 minutes. Media was allowed to cool then poured into petri plates to cover the bottom according to a method by Ezeonu *et al*. (2018).

Appendix 2: Information on synthetic fungicide (AMISTAR TOP)

(Adopted from tps://www.syngenta.co.ke at 1445hrs on 8th August 2018)

Trade mark name: AMISTAR TOP is a broad-spectrum fungicide a product by Syngenta for control of grey leaf spot, leaf blight and common rust in maize. Rust, angular leaf spot and anthracnose in French and common beans. Ascochyta leafspot and powdery mildew on snow peas and powdery mildew in roses.

Active ingredients: 250g/litre Azoxystrobin

125g/litre Difenoconazole

Formulation: soluble concentrates (SC)

WHO classification

II

Mode of action:

Azoxystrobin inhibits spore germination ta early stages of fungal development, this confers excellent protection against invasion by fungalpathogens.it is also active against post germination stages of the life cycle in a broad range of fungal species.it also confers anti-sporulant activity against a wide range of diseases.

Difenoconazole is taken up by the plant and acts on the fungal pathogen during penetration and haustoria formation. It stops development of the fungi by interfering with the biosynthesis of sterols in cell membranes, although the mode of action permits protective and curative use, it is recommended to apply the product early enough to avoid irreversible crop damage and build-up of the disease.

Rate of application: 0.5L/Ha with a 14-day PHI.

Appendix 3: Analysis of Variance (ANOVA) for the effect of *Azadirachta indica, Allium sativum* and *Tithonia diversifolia* crude extracts on spore germination inhibition using methanol, ethanol and distilled water at 50%, 75% and 100% concentrations

Plant material	Treatment	DF	ANOVA SS	Mean Sq	F Value	Pr > F
Allium sativum	Methanol	5	209.12	41.82	237.67	<.0001
	Ethanol	5	254.26	50.85	514.14	<.0001
	water	5	122.56	24.51	426.51	<.0001
Azadirachta indica	Methanol	5	213.72	42.74	201.12	<.0001
	Ethanol	5	253.33	50.67	1383.94	<.0001
	water	5	121.84	24.37	378.46	<.0001
Tithonia diversifolia	Methanol	5	212.83	42.57	229.06	<.0001
	Ethanol	5	259.22	51.84	699.81	<.0001
	water	5	120.70	24.14	384.20	<.0001

Appendix 4: Analysis of variance (ANOVA) table on the effects of *Azadirachta indica*, *Allium sativum* and *Tithonia diversifolia* crude extracts on growth index, disease index and components of yield against *Phaeiosariopsis griseola*

Parameters	DF	Means	Type II SS	Mean Square	F Value	Pr > F
Growth index	4	35.96	344.30	86.07	13.70	<.0001
Pod weights	4	14.50	231.55	57.89	12.65	0.0001
Number of seeds	4	28.80	1601.20	400.30	27.80	<.0001
Fresh weights	4	56.82	5569.27	1392.30	22	<.0001
Dry weights	4	15.65	458.98	114.75	6.70	0.0027
Disease index	4	45.11	10530.91	2632	303.96	<.0001

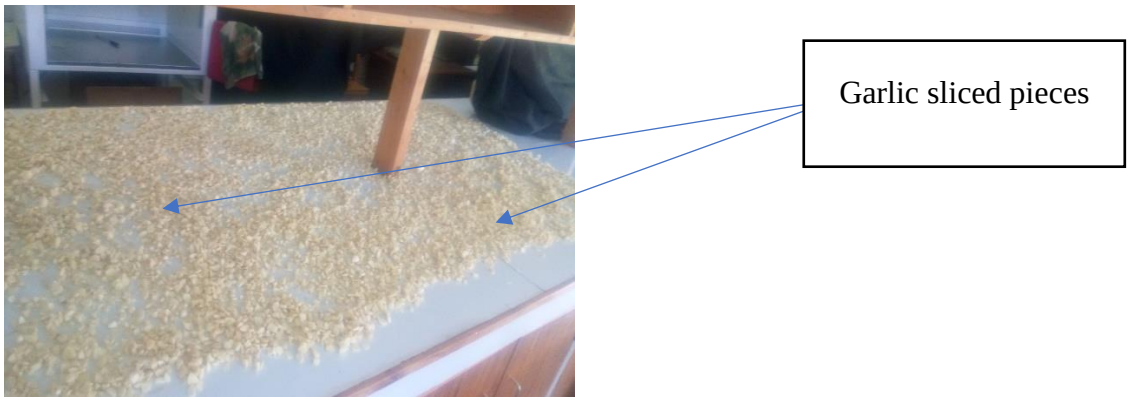
Appendix 5: Minimum Inhibitory Concentration (MIC)

Minimum Inhibitory Concentration of the various methanolic extracts on the fungus, the MIC varied with the extracts used. The MIC values varied from 0.025-0.1mg/ml for the three extracts($P \leq 0.05$). *Allium sativum* extracts had the highest MIC followed by *Azadirachta indica* and the least was *Tithonia diversifolia* extract.

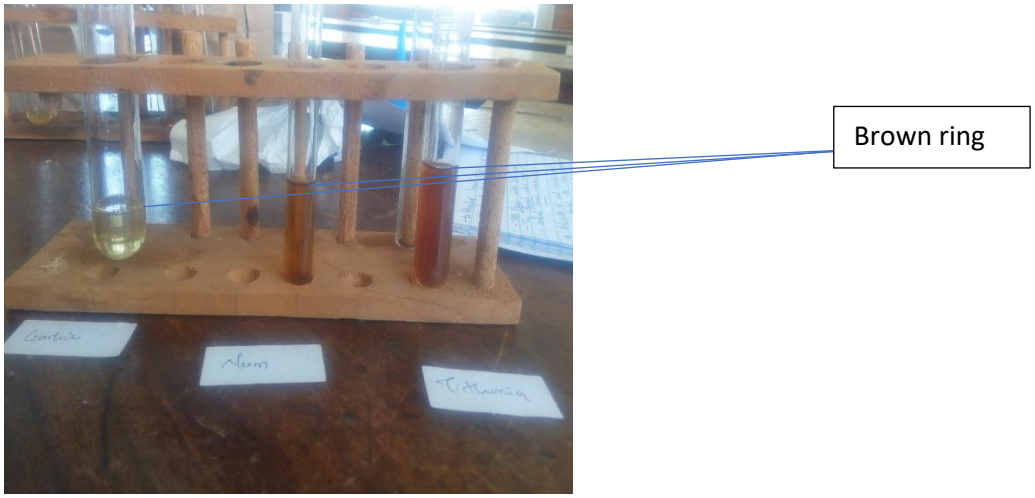
Minimum inhibitory concentration (MIC) of the various methanolic extracts on *Phaeiosariopsis griseola*

Microorganism	**Methanolic plant extracts**	**Minimum Inhibitory Concentration MIC (mg/ml)**
Phaeiosariopsis griseola	*Allium sativum*	0.025
	Azadirachta indica	0.05
	Tithonia diversifolia	0.1

Appendix 6: Garlic drying process



Appendix 7: Phytochemical screening of plant samples: Test for cardiac glycoside that shows interface formation of a brown ring (positive test).



Appendix 8: Grinding of plant material for extraction

Appendix 9: Application of synthetic fungicide using a hand held sprayer in the green house

Appendix 10: Pots containing common bean arranged in a completely randomized design

Appendix 11: Diseased common bean infected with *Phaeiosariopsis griseola* in the green house