EFFICACY OF HOST RESISTANCE, SEED SORTING AND ANTIFUNGAL PLANT EXTRACTS IN MANAGEMENT OF ANGULAR LEAF SPOT OF COMMON BEAN (*Phaseolus vulgaris* L.)

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A thesis submitted to Graduate School in partial fulfilment for the requirement of the Master of Science degree in Plant Pathology of Egerton University.

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DECLARATION AND RECOMMENDATION

Declaration

This thesis is my original work and has not been presented by part or as a whole for examination in any other university.

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To my son Déogratias Marechal Charimbu

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ABSTRACT

Common bean (*Phaseolus vulgaris* L.), is the most important legume in pulses category of Kenya's agricultural commodities and is second only to maize in importance as a food crop. Among the bean diseases angular leaf spot (ALS) caused by *Phaeoisariopsis griseola* is a major constraint to production in the tropics and subtropics, and causes considerable yield losses. The effectiveness of the available methods for control of angular leafspot, which include cultural practices, use of chemicals and resistant varieties is limited by the ability of the pathogen to survive in plant debris for a long period of time, high costs of fungicides, lack of expertise and the health hazards involved, and the high pathogenic variability occurring in *P. griseola*. Therefore, there is need to develop an effective and alternative disease control approach. The strategy most likely to be effective in the control of angular leaf spot is integrated disease management utilizing resistant varieties, natural fungicides and appropriate cultural practices.

In this study 10 bean lines were evaluated for resistance against *P. griseola* under glasshouse conditions. Some of the bean lines were resistant or moderately resistant to various races of ALS pathogen. Bean lines KAB 02-83, KAB 02-9, KAB 12-75 and KAB 02-84 were resistant or moderately resistant to *P. griseola* races 63-63, 63-39 and 63-55. Eight indigenous plants (*Azadirachta indica, Aloe vera, Allium sativum, Warburgia ugandensis, Urtica massaica, Lippea javanicum*, *Tephrosia vogelii* and *Prunus africana)* were tested for their antifungal properties against the angular leaf spot pathogen using paper disc diffusion method. *Allium sativum* showed large inhibition zones on the growth of *P. griseola* culture and reduced the rate of defoliation when sprayed on inoculated bean plants

in the glass house. Seed-to-seedling transmission of *P. griseola* was investigated in order to devise appropriate disease control measures and was found not to be important in the development of ALS. Seed sorting is therefore not effective in management of angular leaf spot. The results of this study indicate that *A. sativum* has antifungal properties against *P. griseola* and can be used as a natural fungicide. Bean lines identified to be resistant to ALS in the glasshouse should be tested under field conditions to ascertain their levels of resistance and recommend them to farmers as an important component of ALS management.

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ABBREVIATIONS AND ACRONYMS

ALS	Angular Leaf Spot
ANOVA	Analysis of Variance
BCMV	Bean Common Mosaic Virus
BLDA	Bean leaf Dextrose Agar
CIAT	Centro Internacional de Agricultura Tropical
GLP	Grain Legume Project
IDM	Integrated Disease Management
KARI	Kenya Agricultural Research Institute
Lsd	Least Significant Difference
m.s.a.l	Metres Above Sea Level
MOA	Ministry of Agriculture
MOALDM	Ministry of Agriculture, Livestock Development and Marketing

CHAPTER ONE

INTRODUCTION

1.1 Bean production in Kenya

Common bean, *Phaseolus vulgaris* L., is the most important legume in the pulses category of Kenya's agricultural commodities (MOALDM, 1996) and is second only to maize in importance as food crop (Gethi *et al*., 1997). It is a major staple food in the diet of people of all income categories, especially as a source of proteins. Bean ranks second as the most important source of human dietary proteins and the third most important source of dietary calories (Pachico, 1993). It is characterized as a near perfect food because of its high protein content and large amounts of iron, folic acid, complex carbohydrates and other diet essentials (Kornegay *et al*., 1996). It plays an important role in controlling the protein calorie malnutrition, a problem that would be highly prevalent in the country since the basic staples are starchy foods.

Bean is predominantly produced in low external input systems by small scale farmers (mainly women). The crop grows under a diverse range of agronomical and environmental conditions, mainly between 900-2700 metres above sea level, and exhibits considerable variation in growth habit and seed type (Acland, 1971; Wortmann *et al*., 1998). Beans do not grow well below 600 m.a.s.l due to the high temperatures which affect pod filling (Stoetzer, 1981). The main bean growing areas are Eastern, Rift Valley, Western, Central and Nyanza provinces, but beans are also grown at the Coast province, mainly in Taita hills (MOA, 1994). The estimated hectarage under beans ranges from 600,000 to 700,000 hectares, producing an annual average of 333,000 tonnes (MOALDM, 1996). The crop is grown either in monoculture or intercropped with other crops such as maize, coffee, bananas, sorghum, millet, potatoes and cassava (MOA, 1990; Mwaniki, 2002; Wachenje, 2002). The most common bean varieties in Kenya include 'Mwezi moja' (GLP-1004), Rosecoco (GLP-2), 'Mwitemania' (GLP-X.92), Canadian wonder (GLP-24), Red harricot (GLP-585), 'Zebra bean' (GLP-806), Rosecoco (GLP-288), Rosecoco (GLP-77), and 'Mwezi moja' (GLP-X.1127A) (Origa, 1992). Small-scale farmers also grow unimproved landraces probably due

to consumer preferences or just die-hard traditional habits (Ogola, 1991; Mwaniki, 2002; Wachenje, 2002).

Bean yields vary greatly, from place to place depending on the climate, soil conditions, seed quality, efficacy of insect pests and disease control and general crop management. The average yields in farmers' fields are generally low; 750kg/ha in monocrop and even lower (375kg/ha) where grown as mixed stand with maize (Njuguna *et al*., 1981; Wachenje, 2002), against a potential of 1500-3000 kg/ha often reported from experimental fields (Rheenen *et al*., 1981; Mwang'ombe *et al.,* 1994). This is far below the yield potential of bush beans which can range from 4,000 to 6,000 kg/ha (White and Izquierdo, 1991).

1.2 Bean production constraints

Typical bean yields obtained in farmers' fields are only 20-30% of the genetic potential of improved varieties (Wortmann *et al*., 1998). These low yields are attributed to a number of biotic and abiotic constraints. Bean is primarily grown by small-scale farmers who have limited resources and usually produce the crop under adverse conditions such as poor agronomic practises, low input use, marginal land, intercropping with competitive crops, low soil fertility, periodic water stress, weed competition and damage caused by diseases and insect pests (Allen *et al.*, 1989; Allen and Edje, 1990; Nderitu *et al*., 1997; Mwaniki, 2002; Wachenje, 2002). Major abiotic constraints include nitrogen and phosphorus deficiency, low pH complex and drought. Severity of root rots and bean stem maggot is aggravated by certain abiotic stresses. Bad weather, lack of improved cultivars and clean planting seeds are also major contributing factors to low yields (Mwang'ombe *et al*., 1994; MOA, 1997; Mwaniki, 2002; Wachenje, 2002). Continuous cropping characterized by minimal or no rotation has led to a decline in soil fertility concomitant with an increase in pest and disease pressure. The major bean diseases include angular leafspot (*Phaeoisariopsis griseola*), anthracnose (*Colletotrichum lindemuthianum*), rust (*Uromyces appendiculatus*), common bacterial blight (*Xanthomonas axonopodis* pv. *phaseoli*), bean common mosaic virus (BCMV), haloblight (*Pseudomonas savastanoi* pv. *phaseolicola)* and root rots caused by a complex of pathogens including *Pythium* sp., *Fusarium solani* fsp. *phaseoli, Rhizoctonia solani, Sclerotinia rolfsii* and *Macrophomina phaseolina* (Buruchara, 1993; Makini, 1994; Mwang'ombe *et al*., 1994; Allen *et al*., 1996). Other diseases such as

Ascochyta blight (*Phoma exigua* var. *diversispora* and/ or *Ascochyta phaseorum)* can also cause significant crop losses, but tend to be confined to specific environments (Buruchara, 1993; Mwang'ombe *et al*., 1994; Wortmann *et al*., 1998). Beans are attacked by a wide range of plant parasitic nematodes but root-knot nematode, *Meloidogyne* spp., are of economic importance (Ngundo and Taylor, 1974; Kimenju *et al*., 1999). The major field insect is bean stem maggot (*Ophiomyia* spp.) while bean bruchids (*Acanthoscelides obtectus* and *Zabrotes subfasciatus*) are rated as important storage pests (Allen *et al*., 1996; Wachenje, 2002). Many of the preferred landraces and popular commercial varieties are susceptible to most of these constraints and are targets of the breeding efforts carried out in Africa, and which often draw upon the wider range of germplasm and traits available from Latin America (Buruchara, 2005). Angular leafspot (ALS) of common bean, caused by the imperfect fungus *Phaeoisariopsis griseola* (Sacc.) Ferraris, is the overall most important constraint in Africa followed (in descending order) by bean stem maggot damage, anthracnose, storage loss from bruchids, common bacterial blight, aphids and BCMV (Allen *et al*., 1996).

Angular leafspot is widely distributed and causes yield losses as high as 80% all over the world (Schwartz *et al*., 1981). In Africa particularly in Kenya, Malawi, Ethiopia, Uganda, Tanzania and the great lakes region, where beans constitute the most important source of dietary protein, ALS is considered the number one constraint to bean production (Pastor-Corrales *et al*., 1998), with annual losses estimated at 374,800 tonnes (Wortmann *et al*., 1998). In Kenya, the disease has been reported in all the bean growing areas (Mwang'ombe *et al*., 1994). When weather conditions are favourable for its development, this disease can be very destructive with crop losses resulting mainly from premature defoliation. The disease affects foliage and pods throughout the growing season and is particularly destructive in areas where warm, moist conditions are accompanied by abundant inoculum from infected plant residues and contaminated seed (Saettler, 1991). In addition to yield losses, the quality, market value and suitability of seed for transport and use across bean-producing regions and national borders may be severely affected (Pastor-Corrales *et al*., 1998).

1.3 Objectives

1.3.1 Broad objective

To screen for host resistance and test the effectiveness of seed sorting and plant extracts in the management of angular leaf spot of beans.

1.3.2 Specific Objectives

1. To screen and select bean germplasm for resistance to angular leaf spot under greenhouse conditions.
2. To determine the level and importance of seed-to-seedling transmission of *P. griseola* in angular leaf spot development.
3. To test the effectiveness of seed sorting in the reduction of angular leafspot development and severity.
4. To test selected indigenous plant extracts for their efficacy in the control of angular leafspot development and seedborne infection.

1.4 Hypotheses

1. High levels of resistance to angular leaf spot exist in some of the bean lines developed by Bean Improvement Programmes.
2. Seed-borne inoculum of *P. griseola* contributes significantly to development and severity of angular leaf spot of beans.
3. Seed sorting is effective in reducing angular leaf spot development.
4. Indigenous plant extracts with antifungal activity can reduce seedborne infection of *P. griseola* and control angular leafspot development.

1.5 Statement of the problem

Angular leafspot disease of beans poses serious challenges in many bean production systems. The disease is present in all bean growing areas of Kenya and was recorded in 89% of the farms visited during a survey of its prevalence, incidence, and severity (Wagara, 2005). Pressure exerted on land by fast increasing human population limits the use of some

cultural disease control practices such as crop rotation or fallowing. Chemical control is hardly a control option for a farmer due to the high cost and the health hazards involved (Wagara, 2005). The problem is further compounded by the fact that small-scale farmers, who are the principal bean growers in Kenya, use their own seed from the previous season or supplement their seed requirements with purchases from informal markets (Mwaniki, 2002). This serves as primary inoculum foci for the development and spread of disease epidemics. Therefore, the strategy, most likely to be effective in the management of ALS is integrated disease management (IDM) involving resistant varieties, natural pesticides and cultural practises.

Use of resistant bean varieties is limited by the wide pathogenic variability occurring in *P. griseola*. Forty four races of the pathogen have so far been identified in Kenya (Wagara, 2005). This high variability dictates that new sources of resistance be continously identified. There is also need to test selected indigenous plant extracts for their antifungal properties against *P. griseola* for use as natural fungicides. Despite the increasing importance of ALS as a constraint in bean production there exists controversy on the importance of seed transmission of *P. griseola* in the development of the disease. Therefore, there is need to study the mode of transmission in order to devise appropriate disease management strategies.

1.6 Justification

This research is justified by the fact that Kenya is an agricultural country and beans play an important role in the country's economy. In addition to being an important food legume, the common bean is a source of income, especially for women who are involved in all aspects of its production and utilization. An increase in bean production is, therefore an important step towards ensuring food security in the country as well as improving the standards of living, especially for the low-income families. To ensure improved and sustainable bean production, effective, economical and environmentally friendly strategies of managing bean production constraints must be developed and used. Identification of bean varieties with resistance to *P. griseola* will play a pivotal role, in combination with other control methods, in the development of an effective intergrated strategy for the ALS disease management.

Use of antimicrobial plant extracts in the control of ALS is an environmentally friendly option because plant extracts are biodegradable and therefore there is no fear of the breakdown products accumulating in the food chain. Determination of the importance of seed-to-seedling transimission of *P. griseola* in the development of ALS will help in developing appropriate disease control strategies aimed at ensuring seed health.

CHAPTER TWO

LITERATURE REVIEW

2.1 Importance of Beans

Common bean is grown for its green leaves, green pods, and immature and/or dry seeds. The dry seeds of *P. vulgaris* L. are the ultimate economic part of the bean plant. They are appreciated throughout the developing world because they have a long storage life, good nutritional properties and can be easily stored and prepared for eating. Dry leaves, threshed pods, and stalks are fed to animals and used as fuel for cooking, especially in Africa and Asia (Celletti *et al.,* 2005). Statistics for dry bean production are vague figures for the biggest producers and consumers in developing countries and are underestimated because beans are often intercropped and/ or grown in remote areas, as a result data is often imprecise. Political disturbances or war sometimes makes statistical analysis difficult or impossible to perform as in the case of DR Congo, Rwanda, and Eastern Europe. Illegal trading also occurs across various borders (CIAT, 2001). In East Africa and Central America, bean is an important staple food crop. Throughout sub-Saharan Africa mostly women farmers grow it traditionally as a subsistence crop. Yet the East Africa Bean Research Network's (EABRN) recent economic surveys show that approximately 50% of producers sell part of their harvest, primarily to urban populations (CIAT, 2001). The income-generating aspect of bean production is becoming more significant principally near urban markets, where populations increasingly rely on bean as an inexpensive source of protein (Correa and Saettler, 1987).

Beans form a significant part of staple diets and play a critical role in human nutrition, providing as much as 45% of total proteins consumed in some areas (Allen *et al*., 1996; Mwaniki, 2002; Wachenje, 2002). One of the major nutritional problems in many developing countries is the lack of sufficient dietary proteins. A substantial part of the population in these areas, mostly the low and medium income families, is not able to obtain the relatively expensive and/or scarce animal proteins. Hence the cultivation of food legumes in such areas is crucial as they offer a cheaper alternative source of proteins (Jalil, 1977). In addition to proteins, beans are a valuable source of minerals (especially potassium,

phosphorous, iron and zinc) and most of B-vitamins (Augustin *et al*., 1981; Nielsen, 1991). Beans also contribute as much as 30% dietary energy in the widespread maize-based cropping systems. Soluble fibre and low fat and sodium content contribute to the suitability of this food in diets aimed at prevention and treatment of coronary heart diseases, in *Diabetes mellitus*, obesity and several colon diseases including cancer (Hughes, 1991). Analysis of dry mature beans gives 22% proteins, 1.6% fat, 57.8% carbohydrates, 4% fibres and minerals for example calcium, 137mg/100g (Kay and Daisy, 1979). Bean is increasingly becoming a significant source of income for small-scale farmers, whether as part of the total farm income or for providing a marketable product at critical times when farmers have nothing else to sell such as before the maize crop is harvested (Wortmann *et al*., 1998). Thus beans play an essential role in the sustainable livelihoods of small holder farmers and their families, providing both food security and income. Because the crop is easily grown, tolerates shade in intercropping and has a growth cycle of only 65-90 days, beans are also important in intensifying agricultural production systems. The whole fresh plant may be ploughed under at flowering stage and used as green manure or harvested and fed to livestock (Mukunya and Keya, 1975). The crop residues are used as animal feed, mulch or manure. Bean plants also habour rhizobium bacterium which fixes free nitrogen from the atmosphere thus maintaining soil fertility (Walker, 1982; Nwokolo and Smart, 1996).

2.2 Distribution of Angular Leafspot of Beans

Angular leafspot is the most widely distributed disease of common bean and has been reported in at least 78 countries (Liebenberg and Pretorius, 1997). Almost all these areas are in the tropical and temperate parts of the world, including Africa, Americas, Europe, Asia, and Australia. Angular leafspot is regarded as the most important disease of dry beans in central and eastern African countries, and in the more humid southern African countries (Danial, 1994; Mwang'ombe *et al*., 1994; Buruchara *et al*., 1995; Wortmann *et al*., 1998). It has been reported in 18 Eastern and Southern African countries and is also considered as one of the most important bean diseases in Central America (Beebe and Pastor-Corrales, 1991; Allen, 1995). A survey conducted to determine prevalence, incidence and severity of angular leaf spot revealed that the disease is widely distributed in Kenya. The disease was prevalent in all the five districts surveyed i.e Kakamega, Kiambu, Embu,

Machakos and Taita Taveta and disease incidence, and severity were generally high (Wagara, 2005).

2.3 Economic Importance of Angular Leafspot

Angular leafspot of beans is a major constraint to bean production in the tropical and subtropical countries (Correa-Victoria, 1988; Saettler, 1991; Mwang'ombe *et al*.,1994; Liebenberg and Pretorius, 1997; Wortmann *et al*., 1998). It is a disease of economic importance in Kenya, especially where ideal conditions for its multiplication prevail (Mwang'ombe *et al*., 1994). When weather conditions for its development are favourable, the disease can cause extensive damage with most crop losses resulting from premature defoliation. Defoliation mainly occurs starting from the flowering and beginning of pod filling stage. Due to reduced foliage, the little food manufactured by the plant is directed towards the up-keep of the plant rather than pod development and filling.

Severe yield losses of upto 80% have been reported worldwide under favourable environment (Correa-Victoria, 1988). Yield losses of more than 50% on susceptible cultivars in India, 80% in Colombia, 51-70% in Brazil and as much as 80% in Costa Rica have been reported (Liebenberg and Pretorius, 1997). In south Africa, annual losses in yields and quality have been estimated to be between 20 and 40% with evidence of losses of upto 80% in certain areas (Boshoff *et al*., 1996). In Pennsylvania, Cole (1966) reported that angular leafspot caused 10-50% reduction in yields with high incidences of small shrivelled beans. In Kenya the disease is considered to be economically important and causes considerable damage on beans (MOA, 1988). However, the precise yield loss due to ALS is often difficult to determine due to frequent occurrence of multiple diseases.

2.4 Nomenclature of *Phaeoisariopsis griseola* (Sacc.) Ferraris

According to Zaumeyer and Thomas (1957), *Phaeoisariopsis griseola* is in the family *Dematiaceae* of subdivision Deuteromycotina, class Hyphomycetes. *Phaeoisariopsis griseola* was first described on *Phaseolus vulgaris* L. in Italy as *Isariopsis griseola* Sacc. in 1878 (Saccardo, 1886). Ferraris (1909) showed that the form-genus *Isariopsis* Sacc. was identical to the previously described form-genus *Phaeoisariopsis* Nob., and renamed the

fungus *P. griseola.* This was recognised by Ellis (1971) and the International Mycological Institute (Anon, 1986). Deighton (1990) distinguished this genus from other cercosporoid fungi on the basis that old conidial scars lie flat against the conidiogenous cells. Other synonyms of this fungus include *Cercospora columnare, C. griseola, Graphium laxum, Isariopsis laxa, Lindaumyces griseola* and *Phaeoisariopsis columnaris* (Liebenberg and Pretorius, 1997).

2.5 Etiology of *Phaeoisariopsis griseola*

2.5.1 Morphological Characteristics of *P. griseola*

Phaeoisariopsis griseola produces clusters of parallel columnal conidiophores known as synnemata that are 20-40µm wide (Liebenberg and Pretorius, 1997). The number of conidiophores to a synnema and the size of synnema show considerable variation. The number of synnemata per lesion range from 5-52 and synnemal conidiophores range from 15-106 (Wagara, 1996). The conidiophores are upto 500µm long, 2-4µm at the base and 5-6µm at the apex. Conidia are formed singly at the tips of conidiophores and are pale olive to olivaceous brown, smooth, obclavate, straight or slightly curved (Ellis, 1971). Significant differences between average conidia length, width and number of septa among isolates have been reported (Buruchara, 1983; Wagara, 1996). Conidia measure 20-80µm in length and 2.85-14.3µm in width. The number of septa per conidium varies from 0-11, with the majority being 2-6 septate (Hocking, 1967; Ellis, 1971; Buruchara, 1983; Wagara, 1996).

2.5.2 Host Range

Phaeoisariopsis griseola infects common bean, *Phaseolus vulgaris* L., whether wild or cultivated, dry or green (snap) and lima bean (*Phaseolus lunatus* L.) (Cardona-Alvarez, 1956). In addition, it also infects scarlet runner bean (*P. coccineus*), tepary bean (*P. acutifolius*), black gram (*Vigna mungo*), pea (*Pisum sativum*), cowpea (*Vigna ungiculata*), hyacinth bean (*Lablab niger*) and *L. purpureus* (Saettler, 1991; Liebenberg and Pretorius, 1997).

2.5.3 Survival and Dissemination

Volunteer plants, off season crops and plant debris have been identified as important sources of inoculum (Sengooba and Mukiibi, 1986). Primary inoculum comes from seed or infested residue The pathogen is reported to survive on infested crop debris over two winters and the stroma that form in lesions allow the pathogen to remain dormant until environmental conditions are favourable for sporulation (Celletti *et al*., 2005). In the absence of the living host, *P. griseola* has been reported to survive for up to 19 months on host plant debris under field conditions (Sindhan and Bose, 1979). Stromata can give rise to spore-forming synnemata for up to 9 months after collection on leaves and 12 months on stems (Cardona-Alvarez, 1956).

Correa and Saettler (1987) retrieved viable samples of *P. griseola* from plants left standing in the field for one winter. Although the viability of conidia declines fairly rapidly, a considerable percentage can survive long enough to infect the crop in the following year. Water and air currents play an important role in dissemination of the fungus. It is possible that viable conidia can be carried over relatively long distances by air currents as beans cultivated on new soil, several kilometres from the nearest known sources, readily became infected under favourable conditions (Liebenberg and Pretorius, 1997). Numerous authors have reported that the pathogen can be isolated from and/or transmitted by means of infested seed (Saettler and Correa-Victoria, 1983; Sengooba and Mukiibi, 1986; Correa and Saettler, 1987). Studies undertaken by Sohi and Sharma (1974) showed that *P. griseola* is borne externally and internally on seed. The hilum and, to a lesser extent, the seed coat surface are sources of contamination. The fungus has been reported to survive on seed for upto 12 months (Sindhan and Bose, 1979).

2.5.4 Symptomatology of Angular Leafspot

This disease affects the foliage and pods of beans in the field during the growing season. It can be a problem when warm moist conditions accompany abundant inoculum from infected plant residues or contaminated seed. All above ground parts are susceptible. Leaf lesions first appear as gray or brown irregular spots having a chlorotic halo. After approximately 9 days, lesions turn brown and necrotic and assume an angular shape

characteristic of this disease. Pod lesions are reddish brown, circular spots, usually surrounded by a darker colored border. On the primary leaves, the lesions tend to be circular but on the trifoliates, the spots are delimited by veins and veinlets, giving them a characteristic angular shape (Saettler, 1991; Wagara, 1996; Liebenberg and Pretorius, 1997). Lesions are often so numerous that they give the foliage a checker board appearance. Under severe infection, lesions coalesce and premature defoliation occurs. The spots appear first on the underside of the leaf and gradually increase as the season progresses. The surrounding tissue may become chlorotic. Hocking (1967) reported the occurrence in Tanzania of a particularly virulent pathotype that formed circular lesion on the trifoliate leaves. Similar pathotypes have been identified among the isolates from Kenya (Wagara, 2005). On pods, symptoms appear as large, circular to elliptical reddish-brown lesions with slightly darker perimeter. Infection may spread to the underlying seeds, which then become discoloured and malformed. On stems and petioles, lesions are dark brown and elongate (Saettler, 1991). Under humid conditions, dark synnemata bearing conidia at the tips are visible in lesions on the abaxial side of the leaves, and may also occur in pod, stem and petiole lesions (Zaumeyer and Thomas, 1957; Saettler, 1991; Wagara, 2005). The atypical isolate described by Hocking (1967) produced conidia on both the abaxial and adaxial surfaces of the leaf.

2.6 Control of Angular Leafspot

The available methods for the control of angular leaf spot can be grouped into three categories; cultural, chemical and use of resistant varieties. Control may be achieved with applications of copper compounds, but the primary and most economical control measures are use of resistant bean varieties and cultural practices (Celletti *et al.,* 2005).

2.6.1 Cultural Control

Plant debris, wind borne inoculum and infected seed are the main sources of infection. Barros *et al*. (1958) reported that ALS was especially serious where beans were planted in or near fields planted with beans during the previous one or more seasons. Other factors contributing to disease severity were close cropping and increased humidity due to irrigation. All dead bean plants should be removed from the garden as soon as possible and planting should be rotated to new locations. A gardener should not save the seed for next

year's use, since *P. griseola* is reported to be seed-borne. One might also consider treating the seed with a dilute Clorox solution (sodium and calcium hypochlorites) before planting (Celletti *et al.,* 2005). Use
of clean seeds, removal of plant debris, crop rotation of at least two years and avoidance of sites adjacent to fields in which beans have recently been harvested can reduce disease severity (Barros *et al*., 1958; Allen *et al*., 1996).

However, the effectiveness of these control strategies is limited due to the ability of the pathogen to survive in plant debris for a long period of time, land unavailability to practise crop rotation and the fact that small scale farmers use their own seeds from the previous season (Isanda, 1994; Mwang'ombe *et al*., 1994; Songa *et al*., 1995; Mwaniki, 2002; Wachenje, 2002). The more practical cultural control measures are the use of cultivar mixtures and intercropping with cereals (Allen *et al*., 1996). In DR Congo, Pyndji and Trutmann (1992) observed that adding of resistant cultivars to farmers mixtures could lead to lower incidences of ALS. A significant reduction in disease incidence was obtained when local and new bean mixtures were supplemented with 25% or more of a resistant line (Trutmann and Pyndji, 1994). Boudreau (1993) observed that intercropping beans with maize can also lead to disease reduction. Mulch treatments are also reported to reduce disease incidence (Mora, 1978), probably as a result of reduction of tansmission due to splashing. The effect of seed sorting has never been tested on the prevalence of seedborne diseases under natural epiphytotics. Among the practices, use of physical seed sorting might be the cheapest and safer method of direct plant disease control (Islam *et al*., 2001). In organic farming physical treatments such as hot water are used to control seedborne pathogens but they involve the risk of seed damage (Mohammad *et al*., 2006).

2.6.2 Chemical Control

Effective use of fungicides results in more than double the yields of bean crop (Liebenberg and Pretorius, 1997). Fungicide evaluation experiments showed that benomyl was the most efficient in inhibiting mycelial growth and decreasing colony dry weight of the ALS pathogen (Sartorato *et al*., 1999). It was further noted that chlorothalonil and fluazinam were the most efficient in the inhibition of conidial germination whereas benomyl showed the best efficiency in ALS control. The fungus can be eradicated from seeds by chemical

treatment of seeds with benomyl at 6g/kg seed and a captan-zineb combination at 3.7g/kg seed (Correa-Victoria, 1984; Saettler and Correa-Victoria, 1985). Elsewhere, Barros *et al*., (1958) found that spraying zineb at 250g/l (1137.5 litres per hectare) five to six times was effective in controlling ALS. Wallace (1952) and Oxenham (1957) used bordeaux mixture (copper sulphate + calcium chloride) and sulphur dust to control the disease. Elsewhere, benzimidazole 60% at 50g/l and trifoline 20% at 200ml/ha sprayed three times in each case were reported to be effective against ALS (Fortugno, 1974). Thiram, carbendazim and captafol are also effective in controlling the disease (Singh and Sharma, 1976; Sindhan, 1984).

However, due to high costs of fungicides, lack of expertise and the health hazards involved, chemical control is hardly a disease control option for the small scale farmers as noted by Wagara (2005). It can only be used to a limited extent, for example in the seed treatment as one of the components of intergrated disease management. Most commercially produced seeds are presently treated with synthetic crop protection agents in order to eliminate seedborne pathogens and protect emerging seedlings from soil and airborne pathogens and insects (Mohammad *et al.*, 2006).

2.6.3 Use of Plant Extracts

Due to the harmful effects of synthetic fungicides, they are only recommended for use as part of an intergrated approach or when other safer methods of control have been found wanting (Islam *et al*., 2004). Use of bio-fungicides is therefore, preferred as a cheaper and environmentally friendly alternative. Several workers have used crude extracts *invitro* to confirm their fungicidal effects against several plant pathogenic fungi (Singh and Prithiviraj, 2002). Extracts from garlic (*Allium sativum* L.) bulb was shown to have fungicidal and inhibitory action on several fungal species e.g *Alternaria* spp. and *Fusarium* spp. (Paul and Sharma, 2002). The efficacy of the leaf extracts of *Azadirachta indica, Cannabis sativa, Aegle marmelos* and *Achyranthus aspera* in controlling leaf spot in faba beans caused by *Alternaria alternata* was determined in laboratory and field experiments conducted in Bihar, India in 2001. *Azadirachta indica* recorded the highest growth inhibition of the fungus under laboratory conditions. Similarly, under field conditions, the crude leaf extract of *Azadirachta*

indica recorded the highest control of the pathogen. Crop yield was highest (40.3 kg/ha) with spraying of *C. sativa* leaf extracts (Singh and Prithiviraj, 2002).

The efficacy of neemazal, a natural product from neem (*Azadirachta indica Juss.*), against pea (*Pisum sativum* L.) powdery mildew (*Erysiphe pisi* DC.) was studied in detached leaf and intact plant experiments. Neemazal significantly retarded several growth parameters of the pathogen, viz. multiple germ tube formation, numbers of germ tubes, branches and haustoria, and colony size on pea leaves. Neemazal induced hypersensitive reaction (HR), as evidenced by browning of host cells associated with appressoria. Furthermore, increase in protein concentration of intercellular fluids followed treatment with neemazal (Singh and Prithiviraj, 2002). Aqueous extract of leaves of neem, provided control of leaf stripe pathogen (*Drechslera graminea*) on barley that was as effective as the fungicide bavistin (carbendazim). The treated leaves exhibited significantly high activity of enzymes phenylalanine ammonia lyase (PAL) and tyrosine ammonia lyase (TAL) along with rapid and distinct accumulation of fungitoxic phenolic compounds (Paul and Sharma, 2002). The leaf pulp of *Aloe vera*, designated as the gel, and the bitter, yellow liquid fraction have been tested against pathogens (bacteria and fungi) affecting human and plants. Antifungal activity of pulp and liquid fraction was evaluated on the mycelia development of *Rhizoctonia solani*, *Fusarium oxysporum*, and *Colletotrichum coccodes* that were isolated from a potato crop by the hyphae point and monosporic techniques. The results showed an inhibitory effect of the pulp of *A. vera* on *F. oxysporum* over a long period (Jasso de Rodríguez *et al.,* 2004). Crude organic extracts of *Warburgia ugandensis*, *Azadirachta indica*, *Tagetes minuta* and *Urtica massaica* were active against all soil pathogens including *Fusarium oxysporum*, *Alternaria passiflorae*, and *Aspergillus niger* (Rugutt *et al*., 2006).

Management strategies of ALS using plant extracts are yet to be investigated as possible alternatives for the control of the disease hence, there is need to screen indigenous medicinal plants for their toxicity to the pathogen. This will provide basic information on formulation of a possible bio-fungicide, which will serve as a better and environmentally safer alternative control measure against ALS in Kenya.

2.6.4 Use of Resistant Varieties

Development and introduction of resistant bean varieties combined with other disease control practises is regarded as the most practical approach of disease control at the farm level. Pyramiding several genes for resistance in one variety is one way of achieving durable resistance in dry bean. One way of achieving this goal involves the testing of the available germplasm and development of improved cultivars with multiple disease resistance (CIAT, 1979). The greatest set-back to this strategy is the high pathogenic variability occurring in the ALS pathogen whereby, a bean variety resistant to some races of the pathogen in one location may be susceptible to other races in the same or different locations. Resistance of beans to angular leafspot has been shown to be of the race-specific type (Mulindwa, 1980) and governed by one, two, or three independent factors (Barros *et al.,* 1957; Cardona-Alvarez, 1958; Santos-Filho *et al.,* 1976; Singh and Saini, 1980). This type of resistance is often complete (Eenink, 1976) but its level may be influenced by genetic dosage effect (Dunn and Mamm, 1970), modifier genes (Rouselle, 1974) or the physiological age of the plant (Bartos *et al.,* 1969).

Sources of resistance to ALS have been identified in *P. vulgaris* and in the secondary and tertiary *Phaseolus* gene pools that include wild *P. vulgaris*, *P. coccineous* and *P. polyanthus* (Mahuku *et al.*, 2002). Efforts are being made to characterize and transfer these genes to well-adapted beans of market types preferred by African farmers and consumers. Some of the bean varieties reported to be resistant in various parts of the world include BAT 332, MEXICO 54, Caraota 260, Cornell 49242 and G5686 (Liebenberg and Pretorius, 1997). Caraota 260 was found to be resistant to 16 out of 18 Kenyan isolates of *P. griseola* tested (Wagara, 1996). It has been reported that the resistant bean accessions, often without any visible ALS symptoms, are the introduced small-seeded genotypes of the Mesoamerican bean races (CIAT, 1996). Barros *et al*., (1958) reported inheritance of resistance to ALS to be recessive and controlled by two or three independent genes, but mentioned that cases of dominant inheritance also occur. Mulindwa (1980) showed that resistance in bean cultivars to *P. griseola* is of race specific type and is governed by a single dominant gene.

There are also indications that rate-reducing resistance against *P. griseola* occurs in some bean cultivars whereby cultivars show differences in the time when symptoms appear and the extent of disease severity (Buruchara, 1983; Wagara, 1996). Reduction of the rate of

infection reduces the epidemic development of a disease by decreasing the reproduction rate of the pathogen (Parlevliet, 1979). The factors of resistance that lead to the latter are the reduction in infection frequency or lesion numbers, lengthening of the latent period and the decrease in sporulation capacity. Very little resistance exists among the large-seeded beans of Andean origin (Liebenberg and Pretorius, 1997). Therefore, gene-pool differences will have to be taken into consideration in the search for sources of resistance to ALS.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Culture and Soil Media Preparation

Bean leaf dextrose agar (BLDA) medium, prepared following the procedure described by Wagara (2005), was used to culture *P. griseola*. One hundred grams of freshly collected bean leaves of Rosecoco GLP-2 were weighed and crushed in a blender with about 100ml distilled water. The mixture was filtered through a double layer of cheesecloth. Fifteen grams of agar and an equal amount of glucose was added and distilled water was added to make one litre. The pH of the mixture was adjusted to 6.8 and the mixture was sterilised in an autoclave at 121^{o}C at 15 psi for 15 minutes. The media was cooled to about 45^{o}C and 133 mg of streptomycin sulphate added. Aliquots of 20ml were dispensed into sterile petri dishes and left to solidify. To prepare agar slants, 15ml of BLDA medium was put in universal bottles and sterilised as above. Once the sterilisation procedure was over, the bottles were removed and placed in a slanting position to solidify. Soil media for greenhouse experiment was prepared by mixing soil, manure, sand and ballast in the ratio of 2:1:1:1. This mixture was sterilised in an autoclave at 100^{o}C, 15 psi for 120 minutes. The mixture was left standing for three weeks to cure and then put in plastic pots (18cmx13cm).

3.2 Culturing and Maintainance of *P. griseola*

Phaeoisariopsis griseola races 63-63, 63-39 and 63-55 collected from Machakos, Embu and Kiambu (Wagara, 2005) were used in this study. Race 63-63 is the most pathogenic, race 63-39 is the most widely distributed and race 63-55 is also highly pathogenic. The pathogen was reactivated from filter paper cultures stored at 4^{o}C by culturing it in BLDA. Small portions of mycelia were picked using a flame-sterilized mounted needle and transferred to BLDA medium. The plates were incubated at 24^{o}C in the dark for 14 days. Agar slant cultures were prepared by point-inoculating the agar slants with mycelia from the 14-day old cultures and they were stored at 4^{o}C for future use.

3.3 Screening of Bean Lines for Resistance to Angular Leaf Spot

Ten bean lines bred for resistance against ALS obtained from the Regional bean breeding program at University of Nairobi were screened for resistance against three races of *P. griseola* (63-63, 63-39 and 63-55) under glasshouse conditions. The bean lines used included; KAB 02-9, KAB 13-13, KAB13-35, KAB 12-24, KAB 02-162, KAB 12-75, KAB 12-39, KAB 02-83, KAB 13-98, and KAB 02-84. Rosecoco GLP-2 and Mex 54 were included as susceptible and resistant checks respectively. In each pot five bean seeds were planted. The greenhouse was partitioned into 3 complete chambers to ensure that each isolate remained into its own chamber to prevent cross infection. The glasshouse had a prevailing temperature range of 16-31^{o}C and relative humidity of 80-95%.

The seedlings were mantained until they were three weeks old and then thinned to three per pot. Three-week old seedlings were inoculated with *P. griseola* isolates at a concentration of 2 x 10^4 conidia/ml by spraying on the upper and lower side of the leaf until runoff using a hand sprayer (East Africa Seed Company). On apperance of the first symptoms, one leaf was tagged per plant and the percentage infected area recorded every two days for 21days. Plants were scored for ALS severity based on a severity scale of 1 to 9 (Schoonhoven and Pastor-Corrales, 1987); whereby 1 = No visible symptoms of the disease, 3 = Presence of few, small nonsporulating lesions covering approximately 2% of leaf area, 5 = Presence of several generally small lesions with limited sporulation covering approximately 5% of the leaf area, 7 = Abundant and generally large sporulating lesions covering approximately 10% of leaf area, 9 = 25% or more of leaf area covered by large sporulating and often coalescing lesions. Plants showing grades 1 to 3 were considered resistant, grade 5 as moderately resistant and those showing grades 7 to 9 as susceptible. The experiment was laid down as a randomized complete design with three replicates and repeated once to confirm results.

3.4 Determination of the Level and Importance of seed-to-seedling transmission of *P. griseola*

3.4.1 Agar and Blotter Test Methods

Angular leaf spot infected bean seeds of Rosecoco GLP-2 were obtained from severely infected pods in farmers' fields and divided into several seed lots of 48 seeds each. One seed lot was plated on bean leaf dextrose agar (BLDA) medium and the second seedlot was placed on moistened sterile filter paper in petri dishes. The plates were incubated at 24^{o}C in the dark and monitored daily for *P. griseola* growth. Resulting colonies were purified and identified and the number of seeds showing infection was determined.

Ten infected bean seeds from the third seed lot were ground into a fine powder and suspended in 5 ml of sterile distilled water. The suspension was serially diluted (three fold) and plated on BLDA medium. To identify the actual location of infection, 24 seeds from the fourth seedlot were soaked in sterile distilled water for 12 hours. The seed coat was aseptically removed, rinsed in sterile water and plated on BLDA medium. The cotyledons were also plated separately and any resulting colonies identified. The experiment was laid down in completely randomized design and was replicated three times. Chi square was used to test the significance of data.

3.4.2 Growing-on Test

Fourty eight infected bean seeds of Rosecoco GLP-2 were potted in 12 polybags with sterile soil (4 seeds per bag) in a glasshouse and the plants monitored for angular leaf spot development. Rate of seed-to-seedling transmission was determined as the percentage number of infected plants. Seeds were harvested and retested for infection as in section 3.4.1 above. Fourty eight clean certified seeds of Rosecoco GLP-2 were surface-sterilised and potted as above to serve as control.

For comparison of the effectiveness of seedborne and artificial inoculum in the transmission of *P. griseola*, certified seeds of Rosecoco GLP-2 (Kenya Seed Company) were surface-sterilised with 2.5% sodium hypochlorite for one minute and rinsed in sterile distilled water twice and potted as above. Three-week old seedlings were inoculated with *P. griseola* at a concentration of 2 x 10^4 conidia/ml and the plants maintained in a glasshouse

until maturity. The seeds were harvested and tested for ALS infection as in section 3.4.1 above. The experiment was laid down in a completely randomized design and replicated three times. The percentage of infected seeds was determined and compared with that resulting from seed-borne inoculum as determined above. t-Test was performed to check statistical significance of the results. The experiment was repeated once to confirm the results.

3.5 Testing the Effectiveness of Seed Sorting in the Management of *P. griseola*

Bean seeds obtained from farmers were 'cleaned' by removing any discoloured, mouldy or shrivelled seeds. Fourty eight 'cleaned' seeds were plated on agar medium (4 seeds per plate) and also potted in sterile soil. Another seed lot (48 seeds) was plated/potted without sorting. The plates were incubated at 24^{o}C and monitored for growth of *P. griseola*. The percentage of seeds showing fungal growth was determined and compared for the sorted and unsorted seed lots. Potted plants were monitored for ALS development. Percentage of infected plants were determined and compared for sorted and unsorted seed lots. The treatments were replicated three times and the experiment was laid down in completely randomized design. Data analysis was done by use of paired t-test and the experiment was repeated once.

3.6 Testing Efficacy of Plant Extracts in Management of *P. griseola*

Extracts of indigenous plants that have been reported to possess antifungal properties against plant pathogens were tested for their antifungal properties against *P. griseola*. These included *Azadirachta indica, Aloe vera, Allium sativum, Warburgia ugandensis, Urtica massaica, Lippea javanicum*, *Tephrosia vogelii* and *Prunus africana* (Oniang'o, 2003; Makeredza *et al.,* 2005; Otanga, 2005).

3.6.1 Aqueous and Methanolic Plant Extraction

Aqueous and methanolic plant extractions were done according to the method described by Otanga (2005). Fresh plant leaves (except for *Allium sativum*) were washed with distilled water and dried at room temperature. One hundred grams of leaves were

ground into a fine powder using mortar and pestle and stirred in 500ml of sterile distilled water to make a homogenous suspension. The suspension was covered with aluminium foil and left to stand for 24 hours at room temperature. The extracts were then filtered through active charcoal to remove chlorophyll. In the case of *Allium sativum*, cloves were cut into small pieces and pound into a thick powder. A homogenous suspension was made by adding 500ml of sterile distilled water and aqueous extraction done as above. Methanolic extractions were done by soaking 100gm ground powders of the plant materials in 500ml redistilled methanol for 24 hours. The suspension were filtered as above and concentrated by rotar evaporation. The extracts were used in the following experiments.

3.6.2 Culture Sensitivity Test of *P. griseola*

Antifungal tests of the plant extracts were done using paper disc diffusion inhibition test according to the method described by Kirby-Bauer (Ching, 1997). Paper discs of 15mm diameter were cut from filter paper (Whatman no 1) and were sterilized in the autoclave at 15 psi 121^{o}C for 15 minutes. The discs were impregnated with the plant extracts and were allowed to air dry on a glass petri dish in the lamina flow hood for two hours. A culture suspension of *P. griseola* (approx 20,000 conidia/ml) was evenly spread over the surface of BLDA solid media in a petri dish and were allowed to dry for about five minutes. Onto this surface, the filter paper discs impregnated with the plant extracts were placed using a flame sterilized forcep. The plates were incubated at 24^{o}C for seven days and observed for inhibition zones. The diameter of inhibition zones was measured and recorded. Treatments were replicated three times and the experiment was laid down in a completely randomized design. The experiment was repeated once and plant extracts inducing large inhibition zones were selected and further tested for their ability to inhibit development of angular leafspot under glasshouse conditions.

3.6.3 Efficacy of Plant Extracts in the Inhibition of Angular Leafspot Development

Plant extracts found to have antifungal properties in the culture sensitivity tests were used to spray seedlings of a susceptible bean variety inoculated with *P. griseola* and evaluation for ALS development and severity done. After germination three weeks old

seedlings of Rosecoco GLP-2 in 14 plastic pots (three seedlings per pot) were inoculated by spraying them with *P. griseola* (20,000 conidia/ml) onto both the upper and lower sides of the leaves using hand sprayer until runoff. After the inoculum had dried up (approx. 30 minutes after inoculation), the plants were separately sprayed with methanolic and aqueous extracts of *A. sativum*. As positive and negative controls plants were sprayed with Ridomil Gold Mz (Metalaxyl 40g/kg + Mancozeb 640g/kg) and distilled water, respectively. All the treated plants were incubated in the glass house and monitored for ALS development. Treatments were replicated three times and the experiment was laid down in completely randomized design. Plants were scored for ALS severity after every two days starting from the seventh day after inoculation, based on a severity scale of 1 to 9 (Schoonhoven and Pastor-Corrales, 1987). The experiment was laid down as a randomized complete design with three replicates and repeated once. A plant extract was considered to have antifungal properties against *P. griseola* if the level of ALS development on the treated bean plants was lower than that of the negative control plants sprayed with distilled water.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Results

4.1.1 Screening of Bean Lines for Resistance to Angular Leafspot

The lesions induced by *P. griseola* races 63-63, 63-55 and 63-39 varied extensively in size and shape depending on the bean line/cultivar and pathogen race. For all the three races the symptoms developed 10-15 days after inoculation. The first symptoms developed on the primary leaves as circular lesions which enlarged and attained larger sizes and in some lines lesions coalesced e.g Rosecoco GLP-2 inoculated with race 63-55 (Plate 1). Race 63-39 induced circular lesions on the trifoliates on beanline KAB 12-75 and the other races induced typical angular leafspots on the trifoliates. In some of the bean lines the symptoms did not develop on the trifoliates. Most isolates induced lesions which were surrounded by chlorotic halos but in race 63-39 there was extensive chlorosis on the leaves e.g KAB 13-35 inoculated with race 63-39 (Plate 2).

Plate 1: Large coalescing lesions of ALS

Plate 2: Intensive chlorosis

Bean line KAB 02-9 showed incompatible host pathogen interaction to some of the races, which were characterized by tiny brown lesions (Plate 3). Cultivar Mex 54 showed complete resistance to race 63-35 (Plate 4). Plants inoculated with races 63-63 and 63-39 showed a slight reduction in the disease severity (Table 1) although race 63-63 is known to be the most virulent of the three races.

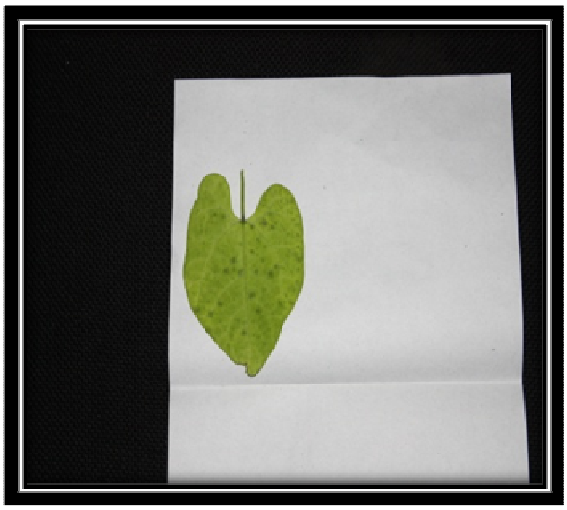

Plate 3: Tiny incompatible brown lesions on bean line KAB 02-9

Plate 4: Culltivar Mex 54 showing complete resistance to *P. griseola* race 63-55

Bean line KAB 02-83 was resistant to race 63-63, bean line KAB 02-9 was resistant to race 63-39, while bean line KAB 02-84 was resistant to race 63-55 (Table 2). Bean lines KAB 02-9 and KAB 02-84 were moderately resistant to race 63-63, while bean line KAB 02-84 was moderately resistant to race 63-39. Bean lines KAB 02-9, KAB 12-75 and KAB 02-83 were moderately resistant to race 63-55.

Table 1: Disease severity scores for *P. griseola* races 63-63, 63-55 and 63-39 on different bean lines under glasshouse conditions

P. griseola races	Bean lines/ Scoring	1[a]	2	3	4	5	6	7	8	9	10	11	12
Race[b] A	1st[d]	1[c]	1	1	1	1	1	1	1	1	1	1	1
	2nd	3	3	7	5	7	7	5	5	5	3	7	5
	3rd	5	5	7	5	7	7	5	5	5	3	7	5
	4th	5	7	9	7	9	9	7	7	7	3	9	5
Race B	1st	1	1	1	1	1	1	1	1	1	1	1	1
	2nd	1	5	5	7	5	7	3	5	7	5	7	3
	3rd	3	5	7	7	7	7	3	7	7	5	7	5
	4th	3	7	7	9	7	9	5	9	7	7	7	5
Race C	1st	1	1	1	1	1	1	1	1	1	1	1	1
	2nd	5	9	7	7	9	5	1	5	7	5	5	1
	3rd	5	9	9	9	9	5	1	5	7	5	5	1
	4th	5	9	9	9	9	7	1	5	9	5	7	3

[a]: indicates Bean lines **1**= KAB 02-9, **2** = KAB 13-13, **3**= Rosecoco GLP-2, **4** = KAB13-35, **5** = KAB 12-24, **6** = KAB 02-162, **7** = cultivar Mex 54, **8** = KAB 12-75, **9** = KAB 12-39, **10** = KAB 02-83, **11** = KAB 13-98, **12** = KAB 02-84.

[b]: indicates *P. griseola* Races A= 63-63, B= 63-39, C = 63-55

[c]: disease severity rating in a scale of 1-9

[d]: 1st, 2nd, 3rd and 4th disease severity scoring

Table 2: Bean lines showing resistance to various *P. griseola* races

Race	Disease reaction	Bean lines
63-63	Resistant	KAB 02-83
	Moderately resistant	KAB 02-9 and KAB 02-84,
63-39	Resistant	KAB 02-9
	Moderately resistant	Mex 54 and KAB 02-84
63-55	Resistant	KAB 02-84
	Moderately resisant	KAB 02-9, KAB 12-75 and KAB 02-83

Bean lines KAB 02-9, KAB 02-83 and KAB 02-84 showed an overall higher resistance to all the three races tested.

4.1.2 Determination of the Level and Importance of seed-to-seedling Transmission

In the blotter paper experiment none of the bean seeds showed growth of *P. griseola*. In the agar method experiment only four out of 48 infected seeds (8%) showed growth of *P. griseola* (Table 3). However, there was recovery of other seedborne pathogens and contaminants which included *Alternaria* spp., *Fusarium* spp. and *Macrophomina* spp.

Table 3: Number of bean seeds showing growth of *P. griseola* from natural seedborne inoculum

	Days	**0[a]**	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Plates	**1[b]**	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	2	2	2
	3	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	0
	5	0	0	2	2	2	2	2	2
	6	0	0	0	0	0	0	0	0

[a]; Days after incubation

[b]; Plate number

None of the 48 plants from seeds obtained from severely infected pods showed development of ALS when potted in the glasshouse but other seedling diseases were observed. Five plants were showing generalized chlorosis and stunted growth. Some seedlings died at early stages of germination and when these seedlings were uprooted there was white mycelia on the roots. When the mycelia was cultured in sterile media the pathogen was identified as *Fusarium solani* fsp *phaseoli*. The death of the seedlings could be due to wilt caused by *Fusarium solani* fsp *phaseoli*. The chi square test showed that seedborne inoculum is not significant in the development and severity of ALS. The graph below (Figure 1) compares disease development from seedborne inoculum and infection after artificial inoculation.

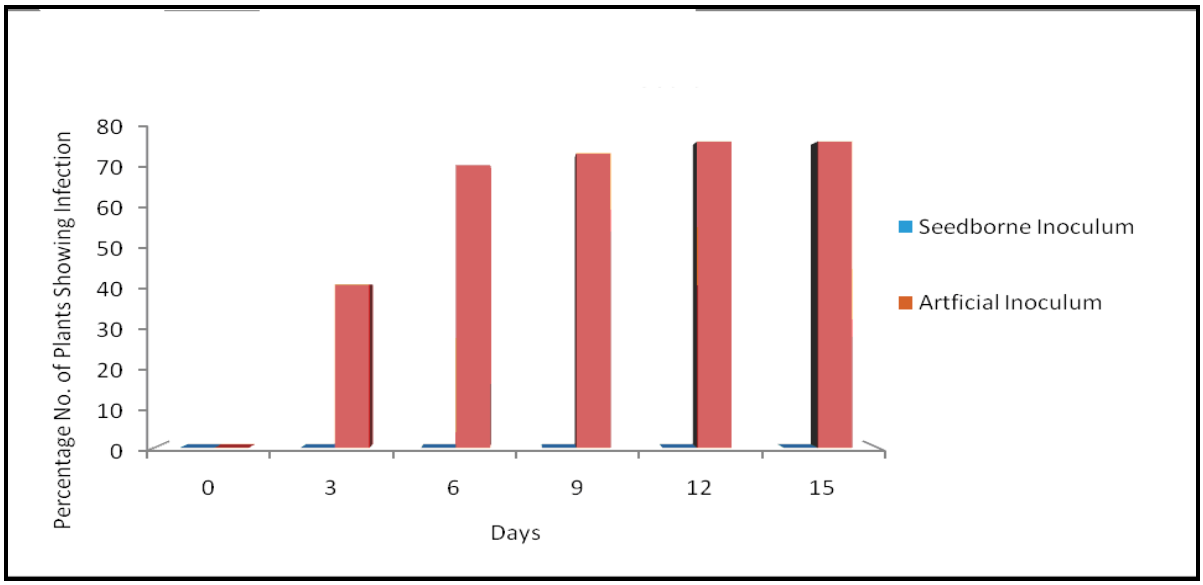

Figure 1: Percentage number of bean plants showing infection from seedborne and artificial inoculum of *P. griseola*.

The highest percentage infection from artificial inoculation of ALS was 77% whereas ALS development from natural inoculum after potting the plants in the glasshouse was 0%.

4.1.3 Testing the Effectiveness of Seed Sorting in the Mangement of Bean Pathogens

After potting and plating of sorted and unsorted bean seeds no seed showed the growth of *P. griseola* which was the pathogen of study and no plant showed development of

ALS. The results provided in Figure 2 below are for the growth of other seedborne pathogens and contaminants which were recovered from the bean seeds. These included; *Fusarium* spp., *Alternaria* spp. and *Macrophomina* spp.

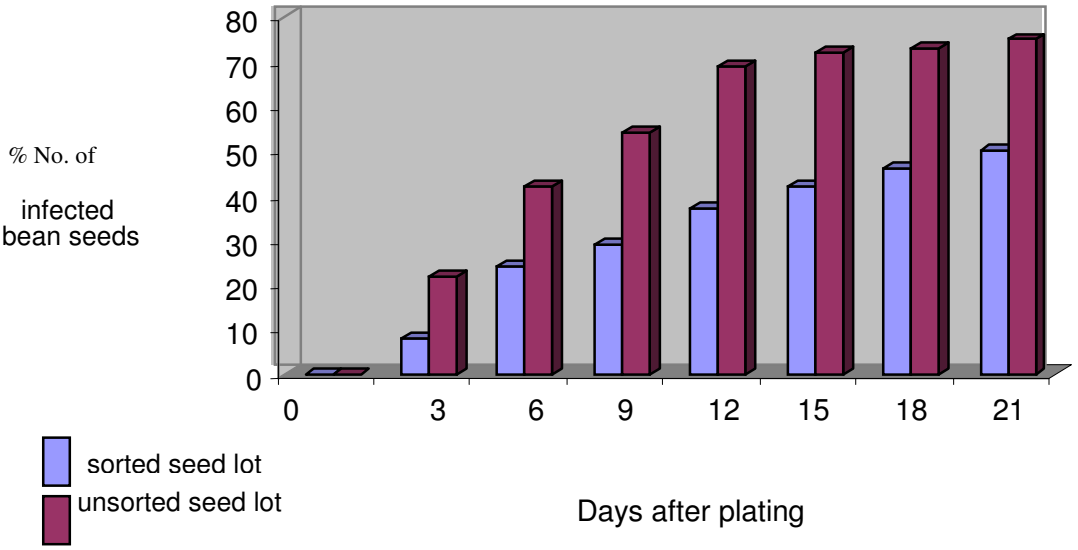

Figure 2: Percentage number of infected bean seeds from sorted and unsorted seed lots plated on BLDA

The unsorted seed lot had a significantly ($P \leq 0.05$) higher percentage of infected seeds as compared to the sorted seed lot. The number of seeds showing fungal growth in the sorted seed lot was still high with the highest showing 50% infection and the lowest showing 8% infection. In the unsorted seedlot the highest percentage number of seeds showing fungal growth was 75%. Statistical analysis confirmed that seed sorting significantly ($P \leq 0.05$) reduced the amount of seedborne inoculum and is important in the management of seedborne pathogens. In addition seed sorting and potting on sterile soil significantly ($P \leq 0.05$) reduced seedling infection as compared to potting the seeds in sterile soil without sorting.

4.1.4 Efficacy of Selected Plant Extracts on Growth of *P. griseola*

Only one out of eight plant extracts evaluated for their antifungal properties against *P. griseola* showed a measurable inhibition zone. *Allium sativum* had the largest inhibition

zone compared to all the other plants evaluated for both aqueous and methanolic extracts (Table 4 and 5).

Table 4: Inhibition zones (in millimetres) of aqueous plant extracts against *P. griseola*

Plant extract	Days after incubation							
	7[a]	8	9	10	11	12	13	14
L. javanicum[b]	0[c]	0	0	0	0	0	0	0
U. massaica	0	0	0	0	0	0	0	0
A. sativum	3	3	2	2.5	1	1	1	1
A. vera	0	0	0	0	0	0	0	0
W. ugandensis	0	0	0	0	0	0	0	0
A. indica	0	0	0	0	0	0	0	0
P. africana	0	0	0	0	0	0	0	0
T. vogelii	0	0	0	0	0	0	0	0
Sterile water	0	0	0	0	0	0	0	0
Ridomil	5	4	4	4	2.5	2	2	2

[a] Number of days after incubation

[b] Plant extracts

[c] Inhibition zones (in millimetres) induced by plant extracts on *P. griseola* cultures

Table 5: Inhibition zones (in millimetres) of methanolic plant extracts against *P. griseola*

Plant extract	Days after incubation							
	7[a]	8	9	10	11	12	13G	14
L. javanicum[b]	0[c]	0	0	0	0	0	0	0
U. massaica	0	0	0	0	0	0	0	0
A. sativum	5	5	4	4	3	3	2.5	2.5
A. vera	0	0	0	0	0	0	0	0
W. ugandensis	0	0	0	0	0	0	0	0
A. indica	0	0	0	0	0	0	0	0
P. africana	0	0	0	0	0	0	0	0
T. vogelii	0	0	0	0	0	0	0	0
Sterile water	0	0	0	0	0	0	0	0
Ridomil	5	4	4	4	2	2	2	1.5

[a] Number of days after incubation

[b] Plant extracts

[c] Inhibition zones in millimetres induced by plant extracts on *P. griseola* cultures

Both aqueous and methanolic extracts of *A. sativum* showed a distinct inhibition zone of 1mm to 5mm (Plates 5 and 6) comparable to the inhibition zone of Ridomil Gold MZ (1.5mm to 5mm) which was used as a standard fungicide.

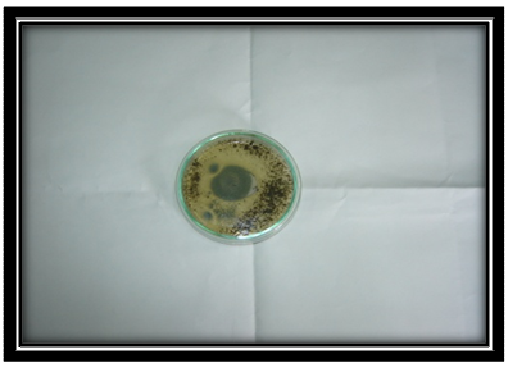

Plate 5: Inhibition zone of *Allium sativum* (methanolic extract) on *P. griseola*

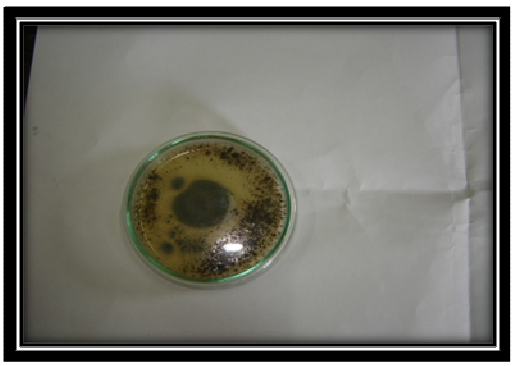

Plate 6: Inhibition zone of *Allium sativum* (aqueous extract) on *P. griseola*

Both aqueous and methanolic extracts of *Urtica massaica* showed no inhibition zones and the pathogen had completely grown on the paper disc impregnated with the extracts (Plates 7 and 8).

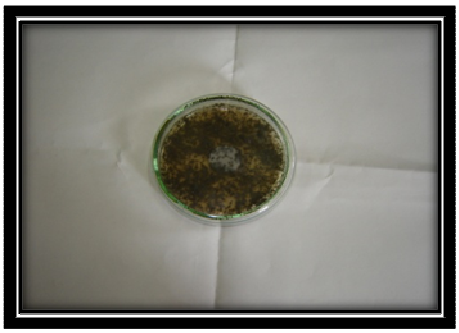

Plate 7: Growth of *P. griseola* showing no inhibition zone by *Urtica massaica* (methanolic extract)

Plate 8: Growth of *P. griseola* showing no inhibition zone by *Urtica massaica* (aqueous extract)

Methanolic and aqueous extracts of *Aloe vera* did not inhibit growth of *P. griseola* but the pathogen did not completely grow on the paper disc (Plates 9 and 10).

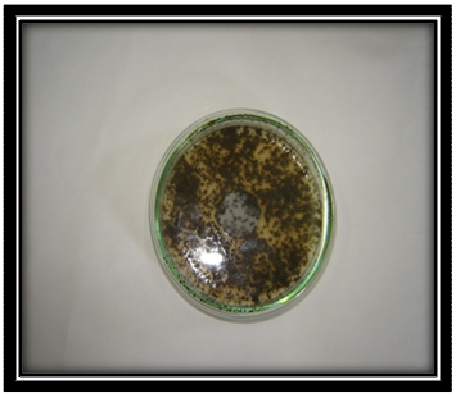

Plate 9: Growth of *P. griseola* showing no inhibition zone by *Aloe vera* (methanolic extract)

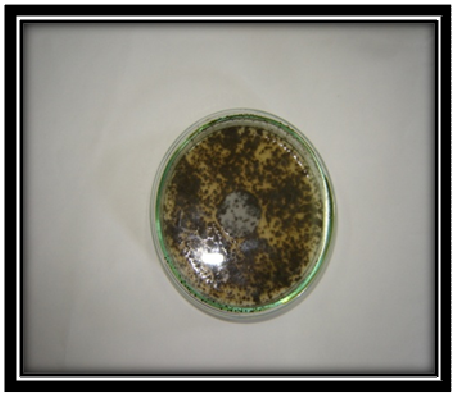

Plate 10: Growth of *P. griseola* showing no inhibition zone by *Aloe vera* (aqueous extract)

Methanolic and aqueous extracts of *Warbugia ugandensis* showed a very minute inhibition zone which was not measurable and *P. griseola* had not grown on the paper disc impregnated with the extract (Plates 11 and 12).

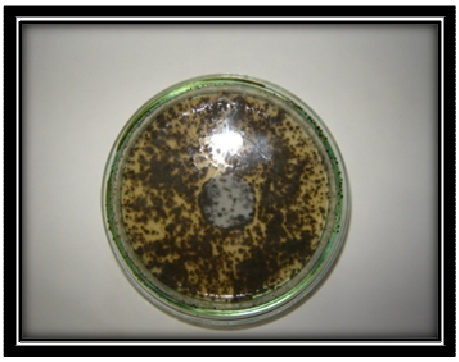

Plate 11: Minute inhibition zone of *Warbugia ugandensis* (methanolic extract) on *P. griseola.*

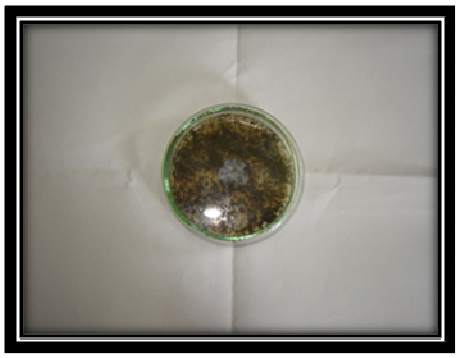

Plate 12: Minute inhibition zone of *Warbugi ugandensis* (aqueous extract) on *griseola.*

Both methanolic and aqueous extracts of *Lippea javanicum* did not inhibit *P. griseola* and the pathogen grew on the impregnated paper disc (Plates 13 and 14).

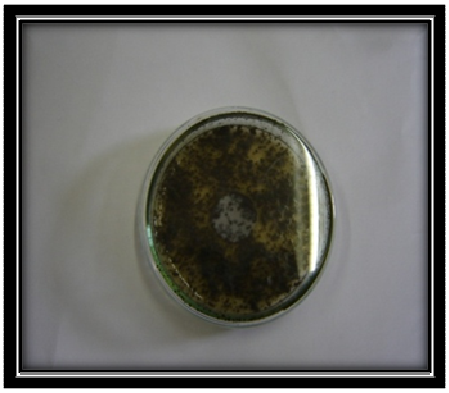

Plate 13: Growth of *P. griseola* showing no inhibition zone by *Lippea javanicum* (methanolic extract)

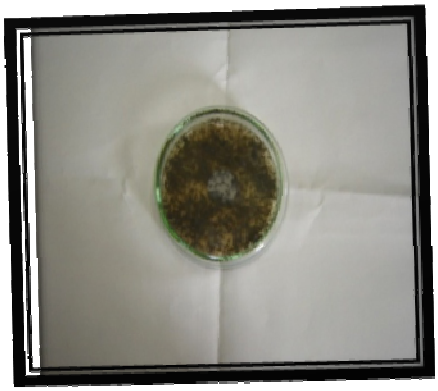

Plate 14: Growth of *P. griseola* showing no inhibition zone by *Lippea javanicum* (aqueuos extract)

Azadirachta indica induced no inhibition zones for both methanolic and aqueous extracts. A sparse growth of *P. griseola* was observed on the paper disc impregnated with the extract (Plates 15 and 16).

Plate 15: Growth of *P. griseola* showing no inhibition zone by *Azadirachta indica* (methanolic extract)

Plate 16: Growth of *P. griseola* showing no inhibition zone by *Azadirachta indica* (aqueous extract)

Methanolic and aqueous extracts of *Tephrosia vogelii* and *Prunus africana* also did not inhibit growth of *P. griseola* (Plates 17, 18, 19 and 20).

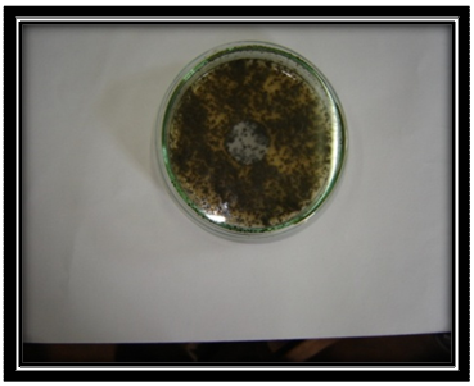

Plate 17: Growth of *P. griseola* showing no inhibition zone by *Tephrosia vogelii* (methanolic extract)

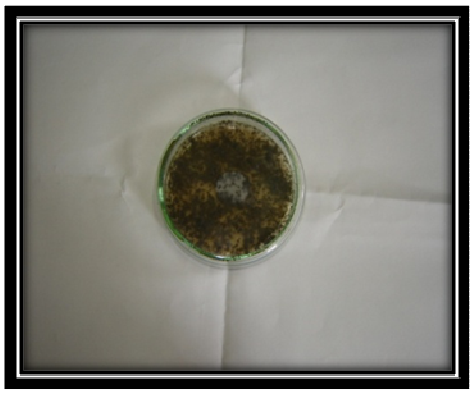

Plate 18: Growth of *P. griseola* showing no inhibition zone by *Tephrosia vogelii* (aqueous extract)

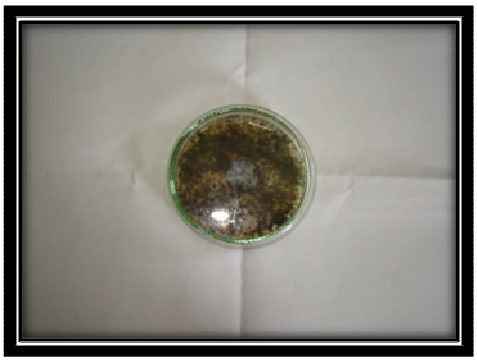

Plate 19: Growth of *P. griseola* showing no inhibition zone by *Prunus africana* (methanolic extract)

Plate 20: Growth of *P. griseola* showing no inhibition zone by: *Prunus africana* (aqueous extract)

4.1.5 Efficacy of plant extracts in the inhibition of angular leafspot development

Aqueous and methanolic extracts of *A. sativum* had no effect on development of angular leafspot. Plants sprayed with the extracts and those sprayed with sterile water to serve as the control had similar disease severity levels (Table 6). However, in the plants which were sprayed with sterile water to serve as the control, angular leafspotg development was accompanied by heavy defoliation (Plate 21) while plants that were sprayed with the extracts had disease development but no heavy defoliation (Plate 22).

Table 6: Angular leafspot scores of bean plants sprayed with methanolic and aqueous extracts of *Allium sativum*

Extract	**Pots/ scoring**	**1[a]**	**2**	**3**	**4**	**5**	**6**	**Control**
Methanolic[b]	1st[c]	1[d]	1	1	1	1	1	1
	2nd	7	7	7	7	9	9	7
	3rd	7	9	9	7	9	9	7
	4th	9	9	9	9	9	9	9
Aqueous	1st	1	1	1	1	1	1	1
	2nd	7	5	7	5	7	5	7
	3rd	7	9	9	7	7	7	7
	4th	9	9	9	9	9	9	9

[a]: Pot number

[b]: Extracts of *A. sativum*

[c]: 1st, 2nd, 3rd and 4th scoring for disease severity

[d]: Disease severity rating scale 1-9

Plate 21: Heavy defoliation in *P. griseola* inoculated plants sprayed with sterile distilled water

Plate 22: No defoliation in *P. griseola* inoculated plants sprayed with *A. sativum* extracts

4.2 Discussion

Bean lines KAB 02-9, KAB 02-84, KAB 02-83 and KAB 12-75 were resistant or moderately resistant to *P. griseola* races 63-63, 63-55 and 63-39. This resistance could be due to resistance genes similar to those in cultivar Mex 54 which is the parent of these bean lines. Several studies have demonstrated that both major and minor genes condition resistance of common bean to *P. griseola* (Singh and Saini 1980., Mahuku *et al*., 2003.) It has been shown that resistance of cultivars AND 277, MAR 2, Cornell 49-242, (*Phg-3*), Mexico 54, and BAT 332 ($Phg\text{-}6^2$) to ALS is due to one dominant gene (Mahuku *et al*., 2003). Allelism tests done by Caixeta *et al*., (2002) revealed that cultivar Mexico 54 has three resistance genes (*Phg-2, Phg-5* and *Phg-6)* to angular leaf spot. The results of this study show that the new bean lines have varying levels of race specific resistance to ALS which could be pyramided to confer durable resistance.

The study on seed-seedling transmission indicates that *P. griseola* is seedborne because about 8% of the seeds showed growth after plating but actual location of *P. griseola* in the seeds could not be identified. This could mean that the methods used in the study were not efficient and other techniques should be used e.g use of staining techniques or use of DNA markers to identify the actual location of the pathogen in the bean seed. The highest percentage infection from artificial inoculation of ALS was 77% whereas from natural inoculum was 0%. Low amounts of *P. griseola* from seedborne inoculum were also reported by Saettler and Correa (1988) where 8 of 20 (40%) and 6 of 59 (10%) seeds were infected. Similar reports were made by Buruchara (2005) where 8 of 250 (3%), 0 of 250 (0%) and 0 of 1000 (0%) seeds were infected. This indicates that seedborne inoculum of *P. griseola* may not be important in the development of ALS but if the environmental conditions are favorable even this small amount of inoculum may cause disease epidemic. Seedling chlorosis and stunted growth observed during the experiment could probably be due to seedborne viral infection.

Seed sorting was found not to be significant in the control of angular leafspot but it is highly significant in the management of other seedborne pathogens From the results on the percentage infected seeds for sorted and unsorted seed lots on both sterile soil and BLDA we can deduce that seed sorting helps to reduce the seedborne pathogens but cannot eliminate all. This can be explained by the fact that seed sorting cannot detect all diseased seeds

because only macroscopic observation is used and some of the seed infection is not visible to the naked eye. Seventy five percent of the seeds from unsorted seedlot showed fungal growth and this could mean that planting seeds without sorting can lead to 75% seedlings being infected. Seed sanitation treatments effective in elimination of pathogens need to be developed (Steven, 2005). Some of the pathogens and contaminants are harboured deep inside the seeds and therefore not visible. In addition some signs of the pathogens and contaminants on the surface may not be visible to the naked eye. Seed sorting, however can be appreciated since it helps to reduce the number of diseased seeds before planting. These results are in agreement with the work done by Steven, (2005) who reported that sorting of barley seed improved their physiological quality by removing less mature seeds and those with the largest fungal infection levels. Seed sorting helps to reduce poor germination and diseased seedling (Steven, 2005). Many serious diseases of pulses can be seedborne and significant crop losses can result from the use of infected seed. Pathogens can adversely affect germination and cause seedling infection or cause damage to mature plants.

Novel seed sorting techniques can be used for removing diseased seeds from contaminated lots or less vigorous seeds. Seedborne diseases often strike early in the plants growth and cause poor crop establishment and reduced plant vigour which results in lower yields and the heavily affected plants can die. This is true since in the present study, in some pots about 58% of the seeds did not germinate probably because their embryo was damaged. Infected seed may sometimes be smaller, shrivelled or discoloured and cleaning and grading may reduce the proportion of such diseased seed. Colour sorting may be used to remove seed that is affected by disease and becomes discoloured. Therefore, seed sorting which is economical, environmentally friendly and saves time and energy spent managing diseases, should be promoted as one of the intergrated methods of managing seedborne diseases.

The results of this study showed that the extracts of *A. sativum* have good inhibitory effects against *P. griseola* but the inhibition zone tends to reduce in size as time passes. This could mean that the active ingredient looses its potency with time. These results are in agreement with the work done by Obagwu and Krosten (2004) who noted that water and ethanol extracts of garlic could control *Penicillium digitatum* and *Penicillium italicum* that causes citrus green and blue mold respectively. *Warbugia. ugandensis* showed some inhibition zone that was not measurable indicating that it has some antifungal activity against *P. griseola*.

Methanolic extracts showed more inhibitory effects than the water extracts, implying that the active ingredients were captured better by methanol. Akunyili *et al.,* (1991) observed similar results when they worked with the stem bark of *Kigelia pinnata* for the management of *Pseudomonas* spp. The failure of water and methanol to extract maximally the active ingredients in the other plants tested in this study may be responsible for the lack of inhibition by these plants. The concentrations used in the study may also have contributed to the lack of inhibition activities.

The reduction in the rate of defoliation after applying the *A. sativum* extracts may be due to the antifungal ability of the extract. These results corresponds with work done by William (2008) who reported that sprays made from aqueous garlic extracts have antibiotic and antifungal properties and will suppress a number of plant diseases, including powdery mildew on cucumbers and to some extent black spot on roses. The antifungal activity of *A. sativum* may be due to sulfur containing compounds such as ajoene and allicin found in it. The effects of antifungal compounds may be on the spore germination leading to its inhibition or may be due to effect of these compounds on the cell wall altering its permeability (William, 2008). The antifungal compounds may also supress the early stages of mycelial growth leading to inhibition of the fungus (Alan *et al.*, 2008). The extracts may also possess growth promoters, hence the continued growth and reduced defoliation in the treated plants despite the high levels of angular leaf spot.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Resistance genes to angular leafspot are present in various bean varieties and lines which have been bred but have not been released for use by farmers. Bean lines KAB 02-9, KAB 02-83, KAB 12-75 and KAB 02-84 were found to be resistant or moderately resistant to races 63-63, 63-39 and 63-55 of *P. griseola* under glasshouse conditions. Seedborne inoculum of *P. griseola* is not important in the development of angular leaf spot but bean seeds are heavily contaminated with other seedborne pathogens which are important sources of primary inoculum.

Allium sativum and *Warbugia ugandensis* possess antifungal activities against *P. griseola* and the bioactive components should be indentified and used for production of natural fungicides. High-performance liquid chromatography (HPLC) can be used to identify the active ingredients. This study provides new scientific information about *A. sativum* based on its antifungal potential against *P. griseola* that has not been reported before.

5.2 Recommendations

1. Beanlines found resistant to angular leaf spot under glasshouse conditions in this study should be tested under field conditions and if found to be resistant they should be released to farmers for use.
2. Seed sorting should be recommended to farmers, since it is cost effective, environmentally friendly and helps save time and energy spent on disease management and in the long run helps improve yields. However, this method is not totally efficient and should be used in combination with other methods such as serological and molecular seed testing techniques in order to ensure disease free seeds. Seed sorting should also be applied together with other integrated disease management strategies for better yields.
3. Extracts of *A. sativum* should be tested for their efficacy in the management of angular leaf spot under field conditions.

4. Further work should look into stabilizing the bioactive ingredient of *A. sativum* so that it does not loose its potency with time.
5. Also there is need to screen other plant parts like the bark, root etc., test higher concentrations of the extracts and use different solvents for extraction. These indigenous plants should also be tested on other plant pathogenic microorganisms for their antimicrobial activities.
6. Further work on the types of phytoconstituents and purification of individual groups of bioactive components can reveal the exact potential of the plant to inhibit several other plant pathogenic microbes. This information can be used in developing a novel broad spectrum antimicrobial herbal formulation.

CHAPTER SIX

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APPENDICES

Appendix 1: Paired statistics for sorted and unsorted seedlots plated on BLDA

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	sorted on BLDA - unsorted on BLDA	-3.0769	1.70595	.47314	-4.1078	-2.0460	-6.503	12	.000

Appendix 2: Paired statistical analysis for development of ALS from sorted and unsorted seedlots and potting in sterile soil

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Sorted seedlot-unsorted seedlot	-37.5000	9.81187	2.83244	-43.7342	-31.2658	-13.239	11	.000

Appendix 3: Scores for angular leaf spot severity for different bean lines inoculated with *P. griseola* races 63-63, 63-55 and 63-39

	Bean lines		1[a]	2	3	4	5	6	7	8	9	10	11	12
Experiment I	Race A[b]	1st[d]	1[c]	1	1	1	1	1	1	1	1	1	1	1
		2nd	1	3	7	3	7	7	1	3	3	5	5	1
		3rd	1	3	7	3	7	7	1	3	3	5	5	3
		4th	1	7	7	7	9	9	7	7	5	5	9	3
	Race B	1st	1	1	1	1	1	1	1	1	1	1	1	1
		2nd	1	5	9	9	7	7	3	7	7	7	7	1
		3rd	3	5	9	9	9	7	3	9	9	7	9	1
		4th	3	9	9	9	9	7	3	9	9	9	9	1
	Race C	1st	1	1	1	1	1	1	1	1	1	1	1	1
		2nd	9	9	9	9	9	9	1	7	7	3	7	1
		3rd	9	9	9	9	9	9	1	7	7	3	7	1
		4th	9	9	9	9	9	9	1	7	7	3	7	3
Experiment II	Race A	1st	1	1	1	1	1	1	1	1	1	1	1	1
		2nd	7	3	7	7	7	7	7	7	7	1	7	7
		3rd	9	7	7	7	7	7	7	7	7	1	7	7
		4th	9	7	9	7	7	7	7	7	7	1	7	7
	Race B	1st	1	1	1	1	1	1	1	1	1	1	1	1
		2nd	1	5	1	7	3	7	5	3	7	5	7	5
		3rd	1	5	3	7	3	7	5	3	7	5	7	7
		4th	1	5	3	9	3	9	7	9	7	5	7	7
	Race C	1st	1	1	1	1	1	1	1	1	1	1	1	1
		2nd	1	9	7	7	9	1	1	5	7	7	1	1
		3rd	1	9	9	9	9	1	1	5	9	7	1	1
		4th	1	9	9	9	9	3	1	5	9	7	1	1

[a]: indicates Bean lines **1**= KAB 02-9, **2** = KAB 13-13, **3**= Rosecoco GLP-2, **4** = KAB13-35, **5** = KAB 12-24, **6** = KAB 02-162, **7** = cultivar Mex 54, **8** = KAB 12-75, **9** = KAB 12-39, **10** = KAB 02-83, **11** = KAB 13-98, **12** = KAB 02-84.

[b]: indicates *P. griseola* Races A= 63-63, B= 63-39, C = 63-55

[c]: disease severity rating in a scale of 1-9

[d]: 1st, 2nd, 3rd and 4th disease severity scoring