Assessment of bean (*Phaseolus vulgaris* L.) cv-Rosecoco GLP-2 seed for infection/ contamination by *Macrophomina phaseolina* and it's implication on disease incidence and severity.

BY

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DECLARATION

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

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DEDICATION

This work is dedicated to my loving husband Mr. G.N. Wakahiu and our children, Wakahiu (Jnr) and Wanjiku.

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ABSTRACT

Charcoal rot, caused by *Macrophomina phaseolina* has been reported to be a major constraint to bean production in Eastern parts of Kenya. Studies were carried out on *M. phaseolina* to determine factors influencing mycelial radial growth under the laboratory conditions to be able to come up with the most ideal incubation conditions for this pathogen. Further studies were carried out to determine the most ideal seed health testing technique for seed borne *M. phaseolina* for the purpose of assessing bean seeds obtained from small scale farmers. Experiments were also conducted to determine the effect of seed borne *M. phaseolina* on incidence and severity of charcoal-rot under field conditions. The influence of bean growth stage on infection of bean plants by charcoal rot and on subsequent seed infection/contamination by *M. phaseolina* was also determined.

PDA (Potato dextrose agar) and MEA (Malt extract agar) supported the fastest growth of *M. phaseolina* at 30-35°C. Lighting conditions did not have any significant (P=0.05) effect on radial mycelial growth.

Direct plating of seeds on PDA revealed significantly (P=0.05) higher infection levels than all the other methods. There was positive and significant (P=0.05) correlation between seed infection levels obtained using dry seed examination and the incubation methods. There was non-significant (P=0.05) correlation between seed infection levels determined by dry seed inspection and incubation methods and the grow-on test.

Seed samples from Kaitha in Machakos district with infection level of 6.5% was noted as the have the highest infection level. Highest losses in germination and post-emergence damping-off were recorded in the plots that were sown with the seed sample that had 6.5% infection /contamination. Incidences of charcoal rot at V3 and R8 were also significantly (P=0.05) high for plots sown with seed sample with infection level of 6.5% when compared to all the other plots. Under field conditions, the seed sample with 6.5% of seedborne *M. phaseolina* had the highest charcoal rot severity. Removal of malformed, discoloured and shrivelled seeds from the seed lot with infection level of 6.5% improved the planting value of the seed leading to low severity. Surface sterilisation after hand sorting did not have any advantage on the subsequent disease

development. Certified bean seeds had the lowest charcoal rot severity. Yield per hactare was not significantly (P=0.05) different among plots sown with different seed samples.

Bean plants inoculated at planting with colonized rice grains had the highest level of subsequent seedborne *M. phaseolina* on harvested seeds at Kiboko. Yield per hectare was significantly (P= 0.05) higher in plots inoculated at maturity and in those plots inoculated with sterile toothpicks at Kiboko. There were no significant (P=0.05) differences in subsequent level of seed infection of seeds obtained from plots inoculated at different stages of growth at Kabete.

CHAPTER ONE

1:0 INTRODUCTION

1:1 Bean production in Kenya

Legumes are among the most important groups of food crops in Kenya. The bean (*Phaseolus vulgaris* L.) is the most important pulse crop in Kenya and is second to maize as a food crop (Acland, 1971). The main bean producing areas are Eastern, Central, Rift Valley and Western provinces of Kenya (MOAMLD, 1994). Beans are also grown in Coast province mainly in Taita Taveta district. In 1992 and 1993 over 502,000 and 628,000 hactares were under beans with yields of over 442,000 tonnes and 408,000 tonnes respectively (MOAMLD, 1994). It is estimated that a total of 800,000 hactares were under bean production in 1994 and this hactarage would raise the annual bean production from 5.3 million to 6.8 million bags (MOAMLD, 1994).

Beans in Kenya are mainly grown by small holders mainly as mixtures consisting of different land races and intercropped with other crops such as maize, sorghum, cowpeas, potatoes, cotton and cassava (Acland, 1971; Mukunya and Keya, 1975). Pure stands occur during the short rains season because the crop is able to utilize limited water supply and reach maturity.

The common bean varieties in Kenya are Rosecoco (GLP-2), Canadian Wonder (GLP-24), Red Harricot (GLP-585), Mwitemania (GLPX-92), Mwezi Moja (GLP-1004) and Zebra (GLP-806) all of which were released by Grain Legume Project of the National Horticultural Research Station, Thika for different Agro-ecological zones (Origa, 1992).

1:2 Beans as an Important Source of Protein

Beans provide a good source of protein to a wide majority of Kenyans. Dry beans contain 20-30% protein with a high concentration of lysine and methionine amino acids (Kinyua, 1980). In addition they contain leucine, minerals and vitamins. Young pods (french beans) of certain varieties are used as green vegetables world wide. Some bean varieties like Mexican-142 are used for canning purposes. In some cases green leaves may

be used as pot herbs or vegetables (Buruchara, 1979; Purseglove, 1987).

Kenya is experiencing fast growth in human population resulting in great demands for food stuffs. Therefore, beans form an important source of proteins to low income earners in towns and the rural populations as animal protein is unaffordable to many (Buruchara, 1979).

Beans are utilized in a number of ways in Kenya. Most people boil them in water and use them as stew with ugali, rice, cassava, chapati and potatoes. They are mixed with maize to form a popular dish for most Kenyans.

1:3 Bean Production Constraints in Kenya

In Kenya, the national bean yield average is well below the potential. The average yield is 500 kg/ha (Mukunya and Keya, 1975; Mutitu and Musyimi, 1980) while the potential is upto 2000 kg/ha. The major production constraint is demand for land for human settlement and competition with cash crops like coffee and horticultural crops. Prices of fertilizers are too high for small scale farmers preventing their use since priority is given to cash crops (Mutitu and Musyimi, 1980). Uneven rainfall distribution, poor cultural practices, destruction by pests and diseases and the unavailability of quality planting seeds has been cited as major constraints to bean production.

Important diseases include Common Bean Mosaic caused by Common Bean Mosaic Virus (Buruchara, 1979), halo blight caused by *Pseudomonas syringae* pv *phaseolicola* (Origa, 1992; Mwang'ombe, *et al.*, 1995), common bacterial blight caused by *Xanthomonas campestris* pv *phaseoli* (Bock, 1970, Acland, 1971; Mukunya and Keya, 1975).

Other major diseases of beans are bean anthracnose caused by *Colletotrichum lindemuthianum*, bean rust caused by *Uromyces appendiculatus*, angular leaf spot caused by *Phaeoisariopsis griseola* Sacc., floury leaf spot caused by *Mycovellosiella phaseoli* (Kinyua 1976/77; Mwang'ombe *et. al.* 1994; Njambere 1997). Charcoal rot caused by *M. phaseolina* is an important disease in the hotter and drier areas under bean cultivation (Songa, 1995). The disease is seed borne and more serious in subtropical and tropical conditions (Andrus, 1938; Neergard, 1973). The disease being seedborne when coupled with heavy seed contamination/infection leads to decay of the seeds in the soil and

causes pre-emergence and post-emergence damping-off (Ludwig, 1925; Kendrik, 1933; Andrus, 1938; Pastor-Corrales, 1986; Songa 1995). Infections later in the growth period result in chlorosis and eventual plant death, severely infected plants do not form pods. (Songa, 1995). In the present study, the disease will be referred to as charcoal rot.

1:4 Sources of Bean Seeds in Kenya

Majority of small scale farmers use their own seed from the preceeding season for planting (Rono and Shakoor, 1990). Seeds may also be purchased from the local markets (Mukunya and Keya, 1975; Buruchara, 1979; Isanda, 1995; Mwang'ombe *et. al*, 1994). Infected/contaminated seeds provide the initial inocula for secondary spread in the field. Introduction of *Macrophomina phaseolina* to the fields through seed would have serious consequences as the pathogen is also soil borne and has a wide host range (Baker *et al*, 1966; Songa, 1995).

Certified bean seed production started in 1975 with the aim of producing high quality seeds (Mukunya and Keya, 1975). The seeds are used locally or exported. Strict regulations concerning seed health are involved in seed export. Samples of bean seeds grown in Kenya for export to Denmark were earlier rejected as they contained *Macrophomina phaseolina* (Richardson, 1979).

Increase in bean yields in Kenya can be achieved through the use of high quality seeds. This can only be achieved by development of sensitive seed health testing techniques for detection of seedborne pathogens. Since charcoal rot is a major disease of beans in Kenya, this project was undertaken to assess the seed borne inoculum and its implications to bean production.

Objectives of the study were:-

1. To determine the factors affecting *in vitro* growth of *M. phaseolina*
2. To determine the levels of seed borne *M. phaseolina* in cv-Rose-coco-GLP-2 seeds obtained from small scale farmers in Kenya.
3. To determine the most ideal seed health testing techniques for *M. phaseolina*.
4. To determine the effect of seed contamination/infection by *M. phaseolina* on incidence and severity of charcoal rot under field conditions.

5. To determine the influence of time of infection of bean plants by charcoal rot on subsequent seed infection/contamination by *M. phaseolina*.

CHAPTER TWO

2:0 LITERATURE REVIEW

2:1:1 Origin and Geographical Distribution of Beans (*Phaseolus vulgaris* L.)

Phaseolus vulgaris L., bean, is thought to have originated from the Mexican regions where it has been grown for the last 4,300 - 6,000 years (Wilsie, 1962). Beans have been growing in Kenya over 300 years (Mukunya and Keya, 1975) after introduction by the Portuguese sailors. Beans are widely cultivated in many parts of the tropics, sub-tropics and throughout the temperate regions (Purseglove, 1987).

2:1:2 Taxonomy of beans

Beans belong to the class dicotyledonae, subclass Rosidae, order Fabales, family Fabaceae or Papilionaceae (leguminosae) and the genus *Phaseolus* (Njambere, 1997). The genus *Phaseolus* include about 31 species of which 10 are cultivated crops (Martin, 1984). *Phaseolus* species are annual or perennial twinning or bushy herbs with trifoliolate leaves, stipules and stipels and typically papilioneous, flowers in axillary or terminal racemes (Purseglove, 1987).

2:1:3 Importance of Charcoal rot

Charcoal rot is a menace to the bean growing industry. Losses start from seed germination failure principally because of rapid decay caused by *Macrophomina phaseolina* in most of the infected seed (Andrus, 1938). High temperatures during pre-emergence phases favours rapid embryo development and reduces seed decay. High temperatures on the other hand during pre-emergence favours the pathogen more than the host resulting in damping-off of the bean seedlings. In later infection, the disease causes stem rots leading to collapse of whole stems. In the United States, the fungus was reported to be of doubtful importance although of wide distribution in the warmer bean growing regions. Later it was found to cause serious losses in hot interior valleys of California where the largest commercial crops of beans are to be found (Ludwig, 1925; Andrus, 1938). *M. phaseolina* is reported to cause bean seedling mortality of 10 - 14 percent in experimental plots and farmers fields in Machakos District (Songa and Rono, 1995). Charcoal rot is

prevalent in Eastern Kenya and is the main limiting factor in the production of common bean. Losses due to the disease are known to exceed 300 kg/ ha in the semi-arid areas of Eastern Kenya (Songa, 1995).

2:1:4 Geographical Distribution of Charcoal Rot Caused by *Macrophomina phaseolina*

Charcoal rot caused by *Macrophomina phaseolina* is a disease widely spread throughout the tropics and subtropical regions of the world. It was noted as being prevalent in warmer areas of United States in the state of California (Ludwig, 1925; Ashby, 1927; Andrus 1938; Kendrick, 1933; Smith, 1969).

Charcoal rot is commonly found in the Indian sub-continent (Mishra *et al.*, 1982; Tiwari and Shroff, 1982; Singh, 1985). It has been reported in Trinidad, Tobago, Myanmer (Burma), Jamaica and Latin America. It has also been reported in Missouri despite extremes of moisture and temperature (Wyllie and Calvet, 1970) and in several African countries (CIAT, 1987).

2:1:5 Taxonomy

Macrophomina phaseolina is classified under the form class Coelomycetes within the mitosporic fungi. The teliomorph is referred to as *Rhizoctonia bataticola*. The fungus is currently referred to as *Macrophomina phaseolina* (Tassi) Goid synonymous to *Macrophomina phaseoli* (Maul) Ashby, *Rhizoctonia bataticola* (Taub) Briton-Jones and *Sclerotium bataticola* (Taub).

2:1:6 Morphological Characteristics

Hyphae is septate, with branched mycelium both superficial and embedded in host tissue giving an ashen-grey appearance. Pycnidia are globose, elongated and ostiolate. Pycnidia measure between 100-250µm, small black sclerotia also produced measure between 50-30µm (Ainsworth, 1976; Alexopoulus and Mims, 1979; Sutton, 1980). The fungus also produces pycnospores on truncate conidiophores of 12-20µm and 6-2µm. Conidia are single-celled, fusiform, measuring 15 - 30 x 5 - 8µm and yellow to reddish brown in colour with a hyaline striale (Thirumalarchar, 1953; Sutton, 1980).

2:1:7 Growth Characteristics

On ordinary PDA media, hyphae of young cultures are white to smoky grey and highly branched with branches arising almost at right angles to parent hyphae. Hyphal branches are constricted at the point of origin (Henson and Valleu, 1937). Generally development of *Macrophomina phaseolina* falls broadly under two categories (Thirumalarchar, 1953), sesamica and typica. Typica group on PDA at 28°C has white mycelia which turns smoky grey. Following the formation of sclerotia the mycelia disintegrate. Sclerotia are uniformly distributed on the medium. Sesamica has white hyphae and turn smoky grey showing concentric zonations of growth. The sclerotia are few and irregularly grouped. The hyphae are not evanescent as in the former type but remain persistent. The fungus produces sclerotia abundantly on liquid media (Abawi and Pastor-Corales, 1986). PDA, carrot agar, Richards medium and onion media gave good sclerotia formation (Aggarwal, 1973; Tiwari and Shroff, 1982; Abawi and Pastor-Corrales, 1986). Songa (1995) obtained abundant sclerotia formation with Czapek dox and rice agar. The pathogen also grows well between 25-30°C with a pH ranging between 6.5 -7. Total darkness, continuous lighting and alternate light and darkness all give good growth (Thirumalarchar, 1953). Sclerotia are important perennating structures of plant fungi (Coleysmith and Cook, 1971) and may be formed in two different ways. The first is by primary branches arising from the main hyphal filament and grow towards each other, secondary branching occurs, filaments intertwine into a loose mass of hyphae. These hyphal strands grow and tend to pile up. The interwoven mass increases in size. Higgins (1927) suggested that interweaving and coalescence is a sexual process involving two different types of hyphae. Willets (1972) observed that the hyphae strands stick together after coming into contact. Buller (1933) found fusions in sclerotia of *Sclerotinia rolfsii* and suggested that this process favours the transportation of nutrients into the developing body. The second way is from a single hyphal strand where branching is initiated from a localised site from a major hyphal filament, several branches occur, proliferate and protrude forming bundle like structures. Hennis *et. al.* (1973) reported that sclerotial formation and hyphae branching are closely related. They reported that substances that inhibit lateral branching

also reduce the sclerotial formation. During the course of development the structures become completely obscured. The structure of mature sclerotia is heavily pigmented, externally covered with mycelial wafts and mucilaginous substances. With age the peripheral cells begin to desiccate leaving cracks and breaks in the cell layers. All the cells retain all the organelles (Wyllie and Brown, 1970). The minimum effective size of a sclerotium is determined by the volume of food base necessary for infection of the host (Akta, 1968).

Several factors influence sclerotial formation. Light induces initiation and development of sclerotia in fungi. Kaiser (1964) found out that in *Verticillium albo-atrum* blue light inhibited microsclerotia production while yellow, orange and red light promoted it. Trevethick and Cooke (1973) found that in *Sclerotinia rolfsii, Sclerotinia delphinii* and *Sclerotinia sclerotiorum* both number and size could be determined by the length of the photoperiods. The most favourable temperature for mycelial growth is usually the best for the formation of sclerotia but this is not necessarily always the case. Goto (1952) reported that in *Sclerotinia rolfsii* sclerotial formation is maximum at or near the optimal temperature for mycelial growth but sclerotia could be formed at temperatures as low at 10°C. He further reported that temperature had an influence on the size and shape of the sclerotia formed. Unfavourable conditions may enhance but are not essential for the formation of sclerotia (Townsend, 1957). Sclerotia initials may be formed on a relatively poor medium but further development requires more nutrients than those essential for the growth of the mycelium (Goto, 1952). He reported that the number of sclerotia formed by *Sclerotinia rolfsii* on agar plates increased with an increasing glucose concentration. However the numbers decreased at higher glucose concentrations. Sclerotia may germinate by a germ tube causing infection and when crushed, they yield oil-like globules. Sclerotia are released in the soil by decaying stems and roots of infected plants (Songa, 1995). They are resistant to extremes of temperature and humidity. High soil moisture reduces the survival of *M. phaseolina* in the soil (Dhingra and Sinclair, 1975). Reduction in activity at increased moisture levels has been attributed to increased bacterial activity, which causes lysis of mycelium and inhibition of sclerotia germination (Bhattacharya and Saadar, 1976). Sclerotia of *M. phaseolina* on bean residues were found to be viable after

21 months in the soil (Songa and Hillocks, 1998) The sclerotia are usually found on the seed surface as contaminant and in internally borne infections, whereby pathogen is usually located in the cotyledons as mycelia (Neergard, 1979).

Pycnidial strains have a great epiphytotic potentiality since the pycnospores are easily dispersed in nature. As opposed to sclerotia, pycnidial strains have been found to be more virulent (Thirumalarchar *et. al.*, 1977). However Songa (1995) observed that pycnidia production was not related to virulence of isolates.

Pycnidial stage is determined by availability of suitable nutritional and environmental conditions. The lower nutrient level in rice agar encourage less mycelial growth but enhance sclerotial production (Songa, 1995). PDA and Czapek dox agar encourage mycelial growth but delayed sclerotial production as they have high nutrient level (Songa, 1995). Pycnidial stage is more common on hosts but Luttrel (1946) reported a pycnidial strain in artificial culture of PDA at 28°C.

2:1:8 Host Range of *Macrophomina phaseolina*

Macrophomina phaseolina has a very wide host range, affecting about 300 plant species. The pathogen affects common beans, potatoes, sorghum, corn, and cowpeas (Andrus, 1938; Hoffmaster *et al*, 1943; Abawi and Pastor-Corales, 1986). It also affects cotton (Tiwari and Shroff, 1982) and linseed (Mishra *et al*, 1982), forest tree nurseries of Douglas fir, sugar pine and giant sequoia (Smith *et al*, 1964).

Charcoal rot has been reported on sorghum, field hyacinth bean, groundnuts, soybeans, green grams and in weed species *Crotalaria* and *Cassia* (Thirumalarchar, 1953; Wyllie and Calvet, 1969; Singh, 1975; Songa, 1995).

2:1:9 Symptomatology of Charcoal rot in beans

Charcoal rot was first reported in 1919 (Andrus, 1938). The term ashy stem blight was proposed in 1926. In seedling stage, damping-off occurs and is characterized by deep black lesions therefore referred to as charcoal rot. In later phase of infection, ashy colour is impacted on stems and petioles by the presence of numerous pycnidia beneath the translucent epidermal layers (Kendrick, 1933). The fungus produces black, sunken cankers

which often contain concentric rings. The plant growing tips may be killed or stem breakage can occur where the stem is weakened by cankers.

Infection may continue to develop into the hypocotyl and root region or on the primary petioles. Older seedling and plant infection cause stunting, leaf chlorosis, premature defoliation and plant death. A few days after infection smooth sclerotia are found on infected tissue and inside plant stems. The fungus may also produce airborne conidia which cause leaf spots on mature plants (Kendrick, 1933).

Seeds contaminated by the pathogen have an ashy grey appearance due to pycnidia adhering to the surface. Numerous pycnidia are found on mature pods and leaves as black bodies (Schwartz and Civillermo, 1979). Ghaffar and Erwin (1969) reported that water stress and hot conditions of 30°C and above enhance disease development. Rain after a prolonged dry spell predisposes plants to the disease. Plants are more susceptible in the productive stage than in vegetative stage.

2:1:10 Pathogenesis

Mycelial and sclerotial inoculum of *Macrophomina phaseolina* is capable of initiating disease in seedlings (Smith, 1969). The sclerotial inoculum is capable of initiating more disease than mycelial inoculum. The sclerotia develop by means of a germtube. Cell wall dissolution is by pectolytic and cellulolytic enzymes (Chan and Sackstom, 1970). Pectin methyl esterase, exopolygluctoranase, endopolygluctoranase and polygluctoranase trans-eliminase have been isolated *in vivo* and *in vitro*. Cellulase and cellobiase activity was also present in cultures. Virulent isolates of the *Macrophomina phaseolina* produce high quantities of endopolygalactronase and exopolygalacturonase. Avirulent isolates produce lower quantities of these enzymes. Once the fungus establishes itself in the tissues it proliferates causing necrosis of the tissues. Numerous pycnidia are formed below the epidermis.

2:2:1 Seed as a Source of Primary Inoculum

The seed transmission of *M. phaseolina* was first demonstrated by Ludwig as early as 1925 and further confirmed later by various workers (Kendrick, 1933; Andrus, 1938; Abawi and Pastor-Corrales, 1986;). They all demonstrated that diseased seedlings could be obtained from seeds that were planted in sterile soil implying that the source of

inoculum was the seed. They further reported that diseased seedlings could be obtained when surface sterilised seeds were planted in sterile soil implying that the infection was deep seated within the seed. Andrus (1938) and Abawi and Pastor- Corrales (1986) demonstrated the presence of *M. phaseolina* on or within bean seed by plating both surface sterilised and unsterilised seeds on potato dextrose agar. *M. phaseolina* may be present on the seed surface as pycnidia or sclerotia as a surface contaminant (Andrus, 1938; Songa, 1995). Numerous black sclerotia carried beneath the seed coat give ashy-grey appearance on the exterior of the seed. Losses due to *M. phaseolina* result from failure of seed germination because of rapid decay by the pathogen. Dipawanita *et. al.* (1994) reported that inhibition of seed germination is by a toxin called phaseolinone and the degree of inhibition of seed germination was correlated well with the amount of toxin produced. Seed germination is a very delicate stage in the life of a plant as it involves the production of young, succulent and constantly expanding tissues with little or no protective cuticle or bark. Andrus (1938) reported that 31% loss in germination due to rapid decay was as a result of the fungus. Environmental factors operating during germination period seem to determine the amount and extent of primary infection. Seedlings may also fail to emerge above the soil due to infection that kills the embryo resulting in pre-emergence damping-off (Andrus, 1938; Abawi and Pastor-Corrales, 1986; Songa, 1995). High temperatures during pre-emergence phase favours rapid embryo development and reduces seed decay. High temperature during post-emergence favours the pathogen and result in damping-off of infected bean seedlings. Infections start from the cotyledonary node and extend to other areas. Once established beneath the seed coat, the fungus is able to penetrate the stem through the base of the cotyledon. The primary leaf become blasted and the fungus proceed through the petiole into the stem.

Conditions favouring adherence of the infected seed coat to the cotyledons favour infection. In turn, conditions favouring adherence of the stem to cotyledons favours infection (Kendrick, 1933). High temperatures (25-30°C) and high humidity possibly account for occasional outbreaks of charcoal rot under field conditions as they favour seedling infection. Infection beyond the primary leaf stage is slower than when infection

occurs before or soon after the plant emerges from the soil. Late infections produce plants that are stunted and wilted. The plants show chlorosis and premature defoliation occurs and finally premature death occur. Losses due to plant death result in poor stand which can result in poor yields. Later infections in the field are characterised by ashy colour on stems and petioles due to the presence of numerous pycnidia beneath the translucent epidermal layer (Kendrick, 1933). Seed transmission introduces the pathogen randomly throughout the field and provides numerous foci for primary infection. Surface sterilised seed coats give sclerotial cultures of *Macrophomina phaseolina* implying that the pathogen resides beneath the seed coat. Seed disinfection was reported to reduce seedling infection by 50% (Andrus, 1938). Abawi and Pastor-Corrales (1986) reported similar had findings on the effect of surface sterilisation.

Andrus (1938) reported that cultures from cotyledons all gave sclerotial cultures of *Macrophomina phaseolina* indicating that the pathogen is located in the cotyledons. Sclerotial cultures of *Macrophomina phaseolina* have been isolated from seeds harvested from symptomless plants and up to 18% infection has been obtained from symptomless pods (Abawi and Pastor-Corrales, 1986).

Beans are affected by many other seedborne fungi (Richardson, 1979) causing considerable losses in terms of quality and yield. *Colletotrichum lindemuthianum* which has a worldwide distribution causes severe losses. The pathogen causes greater losses when badly contaminated seed is planted under conditions favourable for disease development (Isanda, 1995). *Phaeisariopsis griseola* Sacc. which causes angular leaf spot is also seedborne. Infected seeds show a red -brown discolouration from which the pathogen may be transmitted to the seedlings. *Elsinoe phaseoli*, causing scab of beans is also seedborne (Mutitu, 1979). The fungus was introduced with Lima bean seed to the USA (Neergard, 1979).

Fusarium spp. cause root and stem rot. It exists in different species and forma speciales. *Fusarium oxysporum* f.sp. *phaseoli* is prevalent in Kiambu and Murang'a districts causing severe losses (Mutitu, 1989). Other fungi associated with bean seeds include *Botrytis cinerea* which causes grey mould and pod rot, *Cercospora* spp, which causes leaf blotch, *Cladosporium herbarium* which causes *Cladosporium* leaf spot,

Vermatspora carlyli and *Ashbya gossipii* (Richardson, 1979).

2:2:2 Importance of Seed Health Testing in Seed Certification Schemes and Quarantine Regulations

Seed borne pathogens reduce the quality of seeds used for food and sowing for next crops season. Seeds remain viable for a much longer period than do vegetative propagules and this prolongs the potential transmission period and thus the possibility of seed transmission. Pathogens generally remain viable for a long time in association with the seed than they do separately in soil. Seed infection by *Macrophomina phaseolina* lowers germination potential (Andrus, 1938; Abawi and Pastor-Corrales, 1986; Songa, 1995). The seeds decay or fail to emerge above the soil. Seed infection by the fungus lowers potential yields (Neergard, 1979). Introduction of *Macrophomina phaseolina* is dangerous as it is able to persist in field soils and has a wide host range. Once established its extremely difficult to eradicate. The intimate association of host and parasite in the seed provides maximum opportunity for progeny infection.

The seed transmission aspect of *Macrophomina phaseolina* allows spread to previously disease free areas and can introduce new strains of the pathogen to any area (Songa, 1995). Seed certification programmes normally set up tolerance levels for infected seed with the implication that the economic loss from the ensuing diseased plants will be less than the cost of producing seed free from the pathogen or of treating seed to free it from the disease agent (Neergard, 1979). This overlooks the importance of introducing the pathogen or a strain into a new area as well as the rapidity with which some pathogens increase from small initial population. The transmission of seedborne pathogens across boundaries cannot be underestimated. Imported seeds are normally treated with chemicals thereby pathogens are not detected at entry points. However, cumulatively with time, new diseases or strains are reported. For *Macrophomina phaseolina*, it is important to develop routine seed health testing techniques to test seed lots for the presence of the pathogen.

2:2:3 Control of *Macrophomina phaseolina*

Crop yields by farmers are far below those obtained from experimental plots. Mukunya and Keya (1978) estimated potential yield to be 1500 kg/ha. The

seedborne inoculum is most important in epidemic development. Seed dressing with fungicides as slurry treatments have been used widely to control charcoal rot. Chemicals like Thiram, (tetramethyl thiurum disulphide) W.P at a rate of 2.5g formulation per kg seeds has been used (Abawi and Pastor-Corrales, 1986). Khan *et. al.* (1996) evaluated the efficacy of plant extracts and fungicides on mycoflora of chickpea. He reported that the extract from *Calotropis procera* was most effective against all the fungi isolated including *M. phaseolina* and improved seed germination. Among the chemicals, Benomyl (methyl 1-butylcarbomyl) was rated as best followed by Calixin (tridemorph). Comparison of fungicides and plant extracts reveal that *Calotropis procera* was the most effective control. Similarly treatment of jute seed with garlic extract significantly reduced seedborne *M. phaseolina* and improved germination (Khan and Fakir, 1995). Songa (1995) also reported that surface sterilisation with 0.6 percent sodium hypochlorite reduced seed infection to less than 5 percent. The use of chemicals against *Macrophomina phaseolina* is limited as the pathogen has been reported to show tolerance (Anilkumar and Sastry, 1979).

In Kenya, most small-scale bean farmers do not buy certified seeds. They obtain bean seed from their previous crop or from the local market (Schonherr and Mbugua, 1976; Rono and Shakoor, 1990; Origa, 1992; Isanda *et. al.*, 1994; Mwang'ombe *et. al.*, 1995). Much emphasis in the control of charcoal rot is on the use of certified seeds. Selecting clean healthy-looking plants for seed lowers the subsequent seed infection by *M. phaseolina*. Seed selection to remove malformed and blemished seeds has been recommended as a control against many seedborne diseases. Mukunya and Keya (1978) reported that seed selection of bean seeds reduced the disease incidence and severity of bean anthracnose caused by *C. lindemuthianum*. This also holds true for flax infected with *Septoria linicola* (Sackston, 1950) and *Alternaria brassicae* in rape (MacDonald, 1959).

Songa (1995) observed that the level of bean seed contamination /infection was lower after six months of storage than at harvest. Antagonism with other micro-organisms could be a major reason for the inactivation of inoculum in storage. *Aspergillus* spp. was one of the other fungi isolated from the bean seed and has been reported to reduce *Macrophomina phaseolina* infection in peanut kernels (Jackson, 1965).

Cultural practices play an important role in the control of *M. phaseolina*. Removal

of infected crop residues lowers the inoculum levels in infested fields. Deep ploughing and burying crop debris in the soil did not influence the viability of sclerotia and therefore could not be recommended as a control method (Songa. 1995). Crop rotation and intercropping with less susceptible hosts such as chickpea, pigeon pea, groundnut and green gram has been recommended for control (Songa, 1995). Water stress throughout the growth period of the crop should be avoided as water stress predispose the plants towards charcoal rot particularly during flowering. Ghaffer and Erwin (1969) reported that defficiency of soil water aggrevated the severity of charcoal rot in cotton. Schoeneweisis (1975) similary reported that soybean plants that lacked water during flowering had severe attack of charcoal rot.

Surface sterilisation was also reported to reduce the inoculum that is seedborne and has been recommended as a control towards *M. phaseolina* (Songa, 1995). Surface sterilisation with sodium hypochlorite has also been reported to improve germination of brassica seed infected by *Xanthomonas campestris* p. v. *campestris* (Babadoost *et. al.*, 1996). Kabeere *et. al.* (1997) similarly reported lower incidences of *Fusarium graminearum* and *Fusarium subglutinans* from maize seeds that were surface sterilised than those that were non- surface sterilised.

Host plant resistance against *M. phaseolina* has proved to be promising. Beans cultivars like Negrito, Christobal 83, BAT 477 and A55 (Shwartz and Civillermo, 1979; CIAT, 1985) have been identified as being resistant against *M. phaseolina*. Coelho and Dhingra (1996) also identified cultivar EMP 86 as being resistant with IPA 1, Aroana 80 and V8025 as having moderate resistant. Abawi and Pastor –Corales (1986) also identified 15 lines of beans as being resistant. Songa (1995) also identified several cultivars that were resistant against *M. phaseolina*. Breeding for resistant is a long-term process and as such other short term control measures may be preferred in the absence of resistant cultivars.

2:2:4 Time of Infection, weather conditions and growth stage of hosts in relation to seed infection

The degree of infection of seed may be related to the time of infection as defined by the growth stage of the host (Neergard, 1979). The exact time of infection within this

period determines the extent of infection of individual seeds and number of seeds infected (Neergard, 1979). Inoculations carried out later in the growth stage of bean plants with *Pseudomonas syringe* p.v. *phaseoli* (Origa, 1992) and *Colletotrichum lindemuthianum* (Isanda, 1995) had no effect on the level of resultant seed infection.

Isanda (1995) reported that early inoculation of the bean plants with *Colletotrichum lindemuthianum* resulted in significant yield reduction than when inoculations were done late in the growing season. Bolton (1974) and Ayers *et. al.* (1979) similarly reported that maize inoculated with *Drechslera maydis* race T, after the dough stage resulted in the disease with little or no effect on the rate of accumulation or the amount of photosynthate translocated to the grain.

Weather conditions at flowering and seed development are decisive for infection. The amount of seed infection and seed transmission of *Phoma betae* in sugar beet is highly dependent on the amount as well as the time of precipitation. Late precipitation during the harvest leads to high seed transmission whereas early precipitation leads to fairly high percentages of seed infection but only to low rates of seed transmission to the seedlings (Neergard, 1979). Wilson *et. al.*, (1945) inoculated flowering plants of *Lolium perenne* with *Gleotinia temulenta*, causal organism of the blind disease. They found that cool weather conditions favoured the disease by prolonging the formation of apothecia and by increasing the secondary conidial infection. Isanda (1995) similarly carried out inoculations of bean plants with *Colletotrichum lindemuthianum* at different locations and reported that anthracnose was more severe in the location where the weather was cool with high humidity which is necessary for the germination of the spores.

Plants as well as their infectious diseases are generally distributed geographically according to climatic conditions. Climatic conditions leading to severe field attacks by seedborne pathogens usually result in increased seedborne inoculum (Neergard, 1979). *Alternaria* and *Cercospora* diseases prevail in the rainy parts while *Verticillium* wilts and smuts are common in dry climatic conditions (Neergard, 1979). Charcoal rot is prevalent in marginal areas of Kenya (Songa, 1995).

After maturation of the seed, there are possibilities of infection. This may occur during curing of the seed crop while the harvested crop is placed in piles and during

threshing and subsequent processing such as artificial drying and during storage. The entire process may provide conditions favourable for spreading pathogens and saprophytes and for establishment of contamination or infection (Neergard, 1979).

2:2:5 Detection of Seedborne Plant Fungal Pathogens

Direct seed examination, incubation tests on blotter and PDA and grow-on are the methods commonly used in detecting seedborne fungal pathogens (Neergard, 1979). The techniques are standardised methods for the detection of fungal pathogens and basically involve direct examination, incubation and grow-on tests. Generally these methods are relatively easy and do not require sophisticated and expensive equipments (Neergard, 1979). Serological and chemical methods are used to a minor extent. Inspection of dry seed involve examining of seeds for impurities with a low power stereomicroscope (Neergard, 1979). The dry seed inspection is indispensable procedure necessary to supplement to incubation methods (Neergard, 1979). The dry seed examination gives a general idea about the health status of seed samples as seed infection is manifested as shrivelling and discoloration. Andrus (1938) and Songa (1995) working with bean seeds infected by *M. phaseolina* reported that bean seeds infected by *M. phaseolina* have an ashy grey appearance. Soybean infected with purple blotch caused by *Cercospora kikuchi* have a characteristic purple stain (Kilpatrick, 1957). Otieno (1992) reported higher incidences of halo blight caused by *Pseudomonas syringe* p.v. *phaseolicola* on malformed seeds. Isanda (1995) similarly reported that malformed and discoloured seeds had higher incidences of bean anthracnose caused by *Colletotrichum lindemuthianum*. Direct seed examination is an indispensable procedure necessary to supplement incubation procedures but cannot be used independently. Neergard (1979) reported that certain fungi detectable by direct inspection such as *Septoria* on celery may be dead. Low incidences of fungi may not show external signs and symptoms therefore suitable incubation procedures based on development of fungi are imperative to provide full information.

Direct plating of seeds on blotter is relatively cheap and quick method of evaluation of seed health. Recording is also quick and identification of habit characters in each species displaying features such as form, length, size, septation and arrangement of

conidiophores is easy (Neergard, 1979). Identification of *M. phaseolina* is straight forward as sclerotia are formed abundantly on the blotter and on the seed surface. This method can be carried out in poorly equipped laboratories where even basic facilities are lacking. Mathur and Sackston (1963) recommended direct plating of seeds on blotter for evaluation for seeds for infection by *M. phaseolina*. This method is highly recommended, as it is a hybrid between the moist chamber procedure used in plant pathology and the germination test used in seed technology. It provides the advantages of *in vitro* investigations with those of *in vivo* observations (Neergard, 1979). The main limitation of this method is that it does not reveal weak incidences of fungi (Neergard, 1979). Direct plating of seeds on PDA is a very sensitive method. This method can only be carried out in well equipped laboratories. This method is also expensive in terms of media used and more time is required in media preparation. In this method, there is rapid growth of saprophytic and other pathogenic fungi that hamper quick identification of the pathogen.

Grow-on test allows for better symptom development than all the other methods. Neergard (1979) reported that the composition of soil mycoflora may determine the development of pathogenic fungi introduced in the soil with seed. Competition occurs between the organisms on access to nutritional material and the relationship between organisms may positively be dependent, stimulating as in synergism or hampering as in antagonism. Contaminated seed if sown in disinfested soil may lead to more severe development of diseases than contaminated seed sown in untreated soil where antagonistic soil microorganisms are not eliminated. Neergard (1979) reported that *Drechslera sorokiniana*, which is sensitive to the effect of other fungi, developed vigorously in sterilised soil but could almost be suppressed when a little unsterile soil was added.

2:2:6 Seedborne pathogens in relation to disease incidence, severity and yield.

The intensity of disease resulting from seedborne inoculum is of paramount importance in seedborne infection. *Phoma lingam*, the causal agent of black leg of cabbage is found in low incidences in seed but the seedling disease may develop rapidly especially in seedbeds (Neergard, 1979). Low incidences of *Colletotrichum lindemuthianum* in bean seeds result in high outbreaks of bean anthracnose provided the conditions are ideal for the development of the disease (Isanda, 1995). Similarly, low incidences of *Pseudomonas*

syringae p.v. *phaseolicola* can result in high incidences of halo blight (Otieno, 1992). These are examples of diseases with low incidence in seed but a high rate of increase during the growing season. *Septoria apiicola* may have very high inoculum potential in seed but this may lead to weak incidences of disease in the field (Neergard, 1979). This implies that correlation between seed infection percentage and disease intensity is usually variable.

Phoma lingam, the causal agent of black leg of cabbage is reported to cause over 75% reduction in heads even from very low seed infection percentages (Neergard, 1979). Isanda (1995) reported highly significant correlation between seed infection and yield reduction. Neergard (1979) reported no yield differences from different levels of seed infection by *Drechslera caternaria* and *Drechslera dictyoides* in rye grass, *Lolium muiltflorium* and *Lolium perenne*. Crop stand reducers are likely to widen the variation in plant performance in the population. Fernadez *et. al.* (1997) working with *Pyrenophora tritici-repens* on durum wheat reported greater yield per plant derived from red smudge infected seed than non-infected seed in field conditions. Plants are likely to compensate fully for the effect of seedling establishment even over wide ranges. Compensation by adjacent plants may be more important to crop yield in these plants than with average or reduced performance (Gaunt, 1995).

Isanda (1995) reported a negative and significant correlation between disease severity and yield when working with bean anthracnose caused by *Collectotrichum lindemuthianum*. Similarly Sala *et. al.* (1996) reported a 35% reduction in yield by *Sclerotinia sclerotiorum* on sunflower and could use the lesion length of mid stalk rot of infected plants to predict the yield.

CHAPTER 3

3:0 MATERIALS AND METHODS

3:1 Laboratory Experiments

3:1:1 Isolation, culturing, identification and proof of pathogenicity of *Macrophomina phaseolina*

Infected bean stems showing the typical symptoms of charcoal rot caused by *Macrophomina phaseolina* were obtained from the Kabete Campus field station.

3:1:2 Isolation

The infected material was thoroughly washed with tap water and surface sterilised with 1.0% sodium hypochlorite (NaOCl) for five minutes and rinsed with five changes of sterile distilled water. The infected materials were incubated in a sterile petri-dish lined with moist sterile blotter and left on the laboratory bench for 24-48 hours for the fungus to produce sclerotia.

3:1:3 Culturing

Using a hand lens, a single sclerotium was transferred on to potato dextrose agar (PDA) plates and incubated at 28°C for 48-72 hours (Boothroyd, 1967).

3:1:4 Pathogenicity Tests

(i) Planting material

Certified bean seed cv Rosecoco-GLP-2, produced by Hortitec Seed Company and purchased from a seed shop were used for pathogenicity tests as given below.

(ii) Potting medium

The planting medium consisted of soil: manure (cow dung) sand and ballast in the ratio of 2:1:1 1 (Isanda, 1995) by volume and steam sterilised at 80°C overnight and thereafter left to cool for a period of four weeks prior to putting it in black polythene bags.

3:1:5 Inocula preparation and plant inoculation

Two methods of inoculation were used

(i) Colonised whole rice grains

(ii) Toothpick method

(i) Colonised whole rice grains

Inoculum of *Macrophomina phaseolina* can be easily produced on whole rice grains (Abawi and Pastor Corrales, 1986; Songa, 1995). Whole rice grains were kindly supplied by National Irrigation Board (N.I.B.) through the Plant Quarantine Station.

Whole rice grains were autoclaved in water in 1:1 ratio (1 g rice grains to 1 ml of water) and cooled. $1cm^2$ agar blocks were cut from 7-day old cultures of *Macrophomina phaseolina* on PDA and transferred into each beaker containing sterilized rice seeds and incubated at 30°C for 15 days.

The colonised rice grains were used for inoculation during planting by placing three rice grains per bean seed before covering with soil. The potting media was as in Section 3:1.4. The inoculated pots were maintained in a glasshouse and watered daily. Controls were inoculated with autoclaved rice grains. After emergence, the seedlings were observed for symptoms of charcoal rot.

(ii) Toothpick method

Toothpicks were washed with tap water to remove any growth inhibiting chemicals, which could be found on the surface (Dhingra and Sinclair, 1985). The toothpicks were autoclaved at 121°C for 15 minutes in a beaker covered with aluminium foil. Molten PDA was poured aseptically on the toothpicks placed on solidified PDA and the pathogen seeded after media had cooled. The set up was incubated for 14 days at 30°C. Toothpicks covered with micro- sclerotia of *Macrophomina phaseolina* were used for inoculation by insertion just below the cotyledonary node of three-week old seedlings. Sterilised toothpicks were used for inoculating controls and plants were incubated in a glasshouse for three weeks and observed for symptoms of charcoal rot. The plants were watered daily. Isolates from Kabete, Kiambu, Kakamega and Machakos districts were used for pathogenicity tests on bean cultivar Rosecoco-GLP-2. Re-isolation of *M. phaseolina* was done from the inoculated plants so as to fulfil Koch's postulate.

3:1:6 Slide cultures

The slide technique described by Riddel (1950) was used for intact morphological characteristics studies. Two sheets of filter papers, a bent glass rod, microscopic slide on the rod and a coverslip were placed into a petri-dish and sterilised at 180°C for 2 hours.

PDA, which was found to be a good growth medium for the pathogen after preliminary tests, was poured aseptically in a petri-dish to form a 2 mm layer in depth. When the agar had solidified, $1cm^2$ agar blocks were cut and transferred aseptically to a microscope slide. Using a sterile transfer needle, the centre of the block was inoculated using mycelia with sclerotia of the fungus and a coverslip placed centrally on the agar block. To maintain high humidity and to keep the blotter moist, 2-3% aqueous solution of glycerine was periodically added to the petri-dish.

The set-up was incubated at 28^0C for 7 days after which the coverslip was lifted and placed on a microscopic slide to which a drop of lactophenol in cotton-blue had been added. Mounting was also done in clear lactophenol and in sterile distilled water for colour observation of various structures. Examination was done under a Dyzanoom compound microscope at a magnification of 275 times and photographs taken to aid in identification of the fungus.

3:1:7 Pycnidia Production

A method of inducing pycnidial production was adopted from Songa (1995). Water agar (Bactor agar Difco, 1.5%) was poured aseptically in sterile 9cm petri-dishes and allowed to set. Young leaves of growing seedlings of pearl millet (*Pennisetum glaucum* L.) were cut into small pieces (1.5 cm^2), suspended in distilled water and autoclaved at 121^0C for 15 minutes. Two leaf pieces were transferred to the surface of 1.5% water agar medium, in each petri dish after excess moisture had been absorbed on sterile blotting paper. Each of the Petri dishes with leaf pieces was inoculated by transferring a $0.5mm^2$ mycelial disk from the growing margin of 40 hour old pure cultures of the isolate. The inoculated petri dishes were replicated twice and were incubated at 30°C in two sets. One set was incubated at 12 hours of light and 12 hours of darkness and the other one under continous darkness. Observations for pycnidia were made 6 days after incubation under a Dyzanoom compound microscope with a magnification of 400 times.

3:1:8 Effect of growth media, light regimes and temperature on the mycelial radial growth of *M. phaseolina*

These experiments were undertaken to establish the most ideal conditions and medium for growing *M. phaseolina*. Three different types of culture media were used namely PDA (Potato dextrose agar), MEA (Malt extract agar) and BSDA (Bean-seed-decoction agar).

(i) Potato Dextrose Agar (PDA)

Potato dextrose agar 39 g

Distilled water 1 litre

The potato dextrose agar was dissolved in water. The mixture heated to dissolve and then sterilised by autoclaving at 121°C for 15 minutes.

(ii) MEA (Raper and Thom, 1949)

Malt extract agar 20 g

Distilled water 1000 ml

The agar was melted in water and the mixture autoclaved at 121°C for 15 minutes.

(iii) Bean-seed-decoction agar (BSDA)

Agar 20 g

Beans 100 g

Distilled water 1000 ml

100g of beans were boiled in 1 litre of water and strained through cheesecloth. The volume was then adjusted to 1 litre and 20 g of agar (Bacto-Agar-Difco) added. The mixture was autoclaved for 15 minutes at 121°C.

3:1:9 The effect of light and temperature on cultural and morphological characteristics of *M. phaseolina.*

The mycelial radial growth of the pathogen was studied at different temperatures namely 20°C, 25°C, 30°C, 35°C and 40°C on all the three media types namely PDA, MEA and BSDA. The follwing lighting regimes used for each temperature were:-

(a) 24 hours of darkness (24D)

(b) 24 hours of light (24L)

(c) Alternating 12 hours of light and 12 hours of darkness (12L/12D)

Light was provided by two flourescent tubes (cool white, TMX 200) of 240V and 50Hz. Mycelial radial measurements were taken after an incubation period of 48 hours.

3:1:10 Experimental Design and data analysis

All the experiments for cultural studies were carried out using a complete randomized design with three replications. Data was analysed using SAS statistical package (SAS, 1988).

3:1:11 Maintenance of fungal agar slants

Molten PDA was aseptically poured into sterile MaCturney bottles which were fitted at an angle to allow a slant to form. After cooling the slants were inoculated with 36-hour old mycelial/ sclerotial growth of *Macrophomina phaseolina* and the caps loosely closed to allow air circulation. After adequate growth of the pathogen, the bottles were tightly corked. To prevent drying of stored culture, the bottles were flamed at the mouth and cotton wool plug pushed before corking the bottles. The cultures were stored at 4-6^{0}C and used as required upto one year.

3:2:0 Bean seed sample collection from the three rural districts

In Kenya small scale farmers tend to use their own bean seeds for planting and this work was initiated to establish the level of seedborne *M. phaseolina* in farmers' bean seeds.

3:2:1:0 Bean seed sample collection

Samples of *Phaseolus vulgaris* L. cv Rosecoco-GLP-2 were collected from farmers in Kiambu, Kakamega and Machakos districts. The survey was carried out in collaboration with Ministry of Agriculture, Livestock and Marketing officials in Kiambu and Machakos districts. In Kakamega, the survey was carried out with the assistance of Kenya Agricultural Research Institute (K.A.R.I.) officers based at Kakamega Regional Research Centre.

The survey was carried out in December 1995 in Kiambu and in February 1996 in Machakos and Kakamega districts. In each district, 12 sampling areas were randomly chosen. Each sampling area consisted of 8-16 farmers from which 0.5-1 kg of seed samples were purchased.

The samples were put in brown paper bags and clearly labelled. The sub-

samples were mixed to form a representative sample and from which a working sample was drawn according to ISTA rules (Anon, 1985b).

In addition to the above, a commercial seed sample of cv-Rosecoco-GLP-2 was purchased from Hortitec Seed Company. The samples were brought to Plant Pathology Laboratory at the University of Nairobi for seed health analysis. Associated with the representative samples was the problem of dry bean bruchids (*Acanthosclides obectus*) and this was solved by dusting these samples with Actellic (1% Pirimiphos methyl - see (Appendix ii).

3:2:1:1 Assesment of *P. vulgaris* cv Rosecoco GLP-2 seeds from farmers for infection/contamination by *M. phaseolina*

Various methods were employed to assess bean seed obtained from small scale farmers for infection and contamination by *M. phaseolina*. Methods used in this study to detect seedborne *M. phaseolina* were dry seed examination, direct plating on blotter and PDA, plating of surface sterilised seeds on PDA and on blotter and the grow-on test. Analysis of variance to determine the amount of discoloration and malformed seeds was carried out for bean samples from each district.

3:2:1:2 Examination of Dry samples

A low power (4X) stereoscopic microscope was used to assess the seed samples for the presence of sclerotia or ashy grey appearance. Infected seeds were also identified by the presence of an ashy-grey appearance. 100 seeds of cv Rosecoco-GLP-2 in 4 replications arranged in complete randomized design were examined per sample used.

3:2:1:3 Cultural techniques:

Techniques which allowed the actual growth of *M. phaseolina* were used to detect seedborne pathogen. The techniques included blotter and agar plate tests.

3:2:1:4 Plating of non-surface sterilised seeds on blotter and PDA

Petri-dishes measuring 9 cm were lined with three moist blotters soaked in sterile distilled water to which 500 ppm of 2-4-D (2,4-Dichlorophenoxyacetic acid) had been

added. 2-4-D was added to retard seed germination (Harbogs *et. al*, 1950).

In agar plate, bacterial growth was checked by adding 200 ppm of streptomycin sulphate to the molten agar, cooled to 45-48^{0}C just before dispensing into individual plates (Neergard, 1973; 1979).

The seeds were placed at 1 cm apart in each petri-dish, care was taken to prevent the seeds from rolling. The seeds were incubated for 7 days under room conditions. Four replicates each having 100 seeds were incubated for each sample. Experimental design was complete randomized design. A stereoscopic microscope (4X) was used to observe fungal development on bean seeds *in situ*. Seeds infected with *M. phaseolina* were identified by the presence of grey-black mycelia which later disintegrated leaving behind black sclerotia around the seed. The sclerotia were picked using sterile needle and cultured on PDA for confirmation. A Riddel slide culture was also made to confirm the identity of the fungus.

3:2:1:5 Surface sterilisation of bean seeds

Surface sterilisation was done by soaking seeds in 1.0% sodium hypochlorite for three minutes and rinsing with three changes of sterile distilled water. Petri-dishes measuring 9 cm were lined with three moist blotters soaked in sterile distilled water to which 500 ppm of 2-4-D (2,4-Dichlorophenoxyacetic acid) had been added. 2-4-D was added to retard seed germination (Harbog *et. al*, 1950).

In agar plate, bacterial growth was checked by adding 200 ppm of streptomycin sulphate to the molten agar, cooled to 45-48^{0}C just before dispensing into individual plates (Neergard, 1973; 1979). For each sample, four replicates each of 100 seeds were incubated and arranged in a complete randomized design. A stereoscopic microscope (4X) was used to observe fungal development on the bean *in situ*. Seeds infected with *M. phaseolina* were identified by the presence of pale white mycelium. The mycelia later turned grey-black and finally disintegrated leaving black sclerotia around the seed. The sclerotia were picked using a sterile needle and cultured on sterile PDA for confirmation.

3:2:1:6 Grow-on Test (Seedling symptom test)

The experiment was set up to establish the transmission of charcoal rot from seeds to seedlings under glasshouse conditions. Four replicates each containing one hundred seeds from each bean sample were subjected to this test in glasshouse at Kabete Field

Station. Experimental design used was complete randomized design.

3:2:1:6:1 Planting medium

Planting medium was as in section 3:1.4 (ii)

3:2:1:6:2 Planting material

400 seeds from each sample were planted in polythene bags. The seeds were spaced at 3.0cm x 3.5cm and planted 1-1.5 cm deep to give 10 plants per bag. The plants were watered daily to avoid water stress. The glasshouse was sprayed against mites every two weeks using Diazinon (2-Iso-propyl-6 methyl 1-4 Carbamoylmethyl 1-4 pyrimidimyl phosphate) at a rate of 30ml/20 litres of water (40% w/w w.p.)

3:2:1:6:3 Data collection

The number of seeds that had germinated after 14 days was recorded. The number of seedlings with charcoal rot symptoms was recorded. Observations continued for 28 days after planting. Those seeds that did not germinate were exhumed, washed with tap water, surface sterilised with NaOCl (5% w/v), rinsed in three changes of sterile distilled water and plated on PDA to determine the whether they were infected with *M. phaseolina.*

3:2:1:6:4 Data Analysis

Data on seed infection/contamination level was analysed using SAS statistical package (SAS, 1988). Means that were significant were separated using Student-Newman-Keul's Test (Steel and Toorie, 1981).

3:3:0 Determination of the most ideal bean seed health testing techniques for assessing seedborne infection by *M. phaseolina*

There are several methods recommeded by ISTA for detecting seedborne fungi but none has so far been adapted in Kenya for the detection of *M. phaseolina.* The methods evaluated for their effectiveness and convinience in detecting seedborne *M. phaseolina* were dry seed examination, direct plating on blotter and PDA, plating of surface sterilised seeds on PDA and on blotter and the grow-on test.

3:3:1 Ideal seed health testing procedures

Results were adopted from methods described in Section 3:2:1:1 to section 3:2:1:6:4

3:3:2 Data Analysis

Correlation between infection levels by various methods was done and the method that correlated well with the transmission rates was recommended as the ideal seed health testing procedure to be adopted.

3:4:0 Field Experiments

3:4:1 Relationship between seed contamination/infection by *Macrophomina phaseolina* and charcoal rot incidence and severity under field conditions.

After establishing the level of seedborne *M. phaseolina* in each bean sample using laboratory techniques, field experiments were carried out to determine the actual practical implications with respect to transmission of inoculum, establishment and development of disease under field conditions. The major objective of this experiment was to determine the importance of seed-borne *M. phaseolina* on charcoal rot incidence and severity in the field.

3:4:2 Location of experimental fields

The experiment was conducted at two locations namely Kabete Campus Field Station Farm, Faculty of Agriculture, University of Nairobi and at Kiboko Research Station, K.A.R.I. during the long rains from April 1996-August 1996 and October 1996-Jan 1997.

Kabete is about 1800m above sea level and lies within the latitude 1 14' 20"S and 1 15" and the longitude 36 44'E and 36 45' 20"E (Isanda, 1995). On the average Kabete receives about 1046 mm of rainfall per annum with mean temperatures of 23.4^{0}C and 12.6^{0}C (maximum and minimum respectively). The soils are deep, friable clay resistant to erosion (Keya and Mukunya, 1979) with acid humid top soil (humic nitosols developed from Limuru trachyte (Isanda, 1995).

Kiboko is located 170 km South-East of Nairobi on an erosional plain at 1300 m altitude. It is situated on latitude 2°S and longitude 37^{0}E with an average annual rainfall of 621 mm. The soils are deep, dark, reddish brown and dark red friable, clay loam. The soil moisture regime is aridic (ICRISAT, 1992).

3:4:3 Planting material

The seed samples that were planted had different levels of infection/contamination

by *M. phaseolina* as determined by direct plating on blotter. These were -

(a) Seed with infection/contamination of 1%.

(b) Seed with infection/contamination of 4.75%.

(c) Seed with infection/contamination of 6.5%

(d) Hand sorted sample of the seed sample with infection/contamination level of 6.5%.

(e) Surface sterilised of the hand sorted sample seed with infection/contamination sample of 6.5%.

(f) A control sample of commercial seedlot of cv-Rosecoco GLP-2.

3:4:4 (i) Field preparation at Kabete and in Kiboko

Parcels of land measuring about 0.4 ha in size, were ploughed and harrowed. The land was left to settle for one week before beans cv- Rosecoco-GLP-2 were planted. The design used in this experiment was complete randomized block design (CRBD) with six treatments replicated four times. Before planting, twenty-four plots measuring 2m x 4m were marked out in the field with each plot or block separated from each other by a distance of 1m.

Planting rows were marked 50cm apart in various plots into which Diammonium phosphate (DAP) fertilizer was applied at a rate of 1kg/100m row length. Cv-Rosecoco-GLP-2 seeds were planted with a spacing of 10cm between plants and guard rows were planted all round using bean line L23.

Fourty- two plants were planted per line with 5 rows per plot to give 210 plants per plot to give a crop density of 260,000 per ha. At Kiboko site, overhead sprinkler irrigation was applied every week (ten times) throughout the growing season. At Kabete site, overhead sprinkler irrigation was done (five times) when the rainfall was insufficient.

(ii) Weed and pest control

For the control of bean stem maggots, Diazinon, (2-Iso-propyl-6 methyl 1-4 Carbamoylmethyl 1-4 pyrimidimyl phosphate (Murphy chemicals)) was applied at a rate of 80ml/20 litres of water soon after 12 days from planting and subsequently after fourteen

days (40% w/w w.p.). Plots were kept weed-free by hand cultivation throughout the cropping period.

3:4:5 Data collection

3:4:5:1 Germination

The number of seedlings emerged two weeks after planting was calculated as a percentage of the total seeds sown.

3:4:5:2 Post-emergence damping-off

The number of emerged but dead seedlings (damping off) was recorded and expressed as a percentage of the total seeds sown.

3:3:5:3 Charcoal rot incidence

Incidence was recorded as number of plants showing charcoal rot symptoms at V3 and R8 and expressed as a percent of the total number of seeds sown.

3:3:5:4 Charcoal rot severity

Severity was recorded every two weeks on basis of twenty randomly labelled plants. The same plants were assessed for the charcoal rot severity throughout the entire growth period. The charcoal rot severity was assessed on stem and was based on C.I.A.T. scale (CIAT, 1987) using the percent infected stem with respect to the stem length area (Table 1).

3:4:5:5 Yield data

The plants were left to dry completely in the field before threshing them. Each plot was harvested and the total yield for each treatment was converted to yield per hactare.

3:4:5:6 Percentage yield reduction data

Percentage deviation in yield due to treatment was calculated using the formula below (Isanda, 1995)

$$\% \text{ loss in yield due to seed infection} = \frac{\text{Yield in control} - \text{Yield due to infection}}{\text{Yield in control}}$$

3:4:5:7 Data Analysis

Data on germination, post emergence damping-off, incidences at V3 and R8 was transformed using square root transformation (X+1) (Steel and Toorie, 1981) before analysis. Data on severity and yield was not transformed. Data analysis was analysed using SAS statistical package (SAS, 1988). Data was compared statistically by analysis of variance. Significant ranges were separated using Least significance difference (LSD). Correlation between seed infection, severity and yield were also worked out.

Table 1. Charcoal rot severity evaluation key (C.I.A.T, 1987)

1. No visible symptoms
2. Very tiny lesions on the stem
3. Charcoal rot symptoms are restricted to the cotyledons. Lower stems tissues are covered with small and superficial lesions.
4. Approximately 5% of the hypocotyl and lower stem tissues covered with lesions and often combined with fruiting structures of the fungus.
5. Approximately 10% of the hypocotyl and lower stem tissues covered with lesions and often combined with fruiting structures of the fungus.
6. Approximately 20% of the hypocotyl and lower stem tissues covered with lesions and often combined with fruiting structures of the fungus.
7. Approximately 25% of the hypocotyl and lower stem tissues covered with lesions and often combined with fruiting structures of the fungus.
8. Approximately 35% of the hypocotyl and lower stem tissues covered with lesions and often combined with fruiting structures of the fungus.
9. Approximately 50% or more of the hypocotyl and stem tissues covered with large number of fruiting structures of the fungus.

3:5:1 Relationship between time and level of charcoal rot infection of cv-Rosecoco-GLP-2 plants and the resultant seedborne *M. phaseolina* in harvested seeds.

The main objective of this experiment was to establish the critical time of charcoal rot infection on bean plants that would result in any detectable *M. phaseolina* on harvested seeds. Once established, the concept could be used in disease control programme for bean seed production in Kenya.

3:5:2 Location of experimental site and design

The experiment was carried out at Kiboko Research Station and in Kabete Campus Field Station Farm on parcels of land that had not been planted with beans for a period of 3 years. The plots were sown with certified seeds and inoculated at different growth stages in the long rains season (April 1996 to August 1996) and in short rains (October 1996 to January 1997). The trial consisted of three replicates in a complete randomized block design. Fourty- two plants were planted per line with 5 rows per plot to give 210 plants per plot to give a crop density of 260,000 per ha. At Kiboko site, overhead sprinkler irrigation was applied every week (ten times) throughout the growing season. At Kabete site, overhead sprinkler irrigation was done (five times) when the rainfall was insufficient.

3:5:3 Field practices

Field practices were carried out as in section 3:3:2

3:5:4 (i) Inoculation of bean crop with *M. phaseolina*

The plants were inoculated at different developmental growth stages and the treatments were as follows:-

1) Seeds inoculated with colonized rice grains at planting.

2) Colonised toothpick inoculation at seedling stage (2 weeks) after planting (1st trifoliolate leaf).

3) Colonised toothpick inoculation at 4 weeks after planting.

4) Colonised toothpick inoculation 6 weeks after planting

5) Colonised toothpick inoculation at pod filling stage (8 weeks after sowing), i.e., when 50% of the plants had put on pods.

6) Colonised toothpick inoculation at mid pod maturity

7) Control:- inoculated with sterile toothpicks two weeks after sowing.

3:4:5:0 Data collection

3:4:5:1 Yield Data

Data was taken as in section 3:4:5:5

3:4:5:2 Data analysis

Analysis of variance was carried out on data on yield using SAS statistical package (SAS, 1988). Means were separated using Least significance difference (Steel and Toorie, 1981).

3:4:5:3 Seed Health Testing

The harvested seeds were evaluated for the presence of seedborne *M. phaseolina*. From each treatment, seeds were bulked to form a single representative sample from which 400 seeds were randomly taken. The procedure used for the detection of seedborne *M. phaseolina* was the blotter method described in section 3:2:1:4.

3:4:5:4 Data analysis

As in 3:2:2:4. The correlation coefficient between the infection level of the harvested seed and the stage at which inoculation was carried out was also worked out.

CHAPTER FOUR

4:0:0 RESULTS

4:1:1 Morphological characteristics of *M. phaseolina*, the charcoal rot pathogen.

(i) *M. phaseolina* on infected bean tissue.

After 24 hours of incubation, black mycelium embedded in the host tissue could be seen. After 48 hours of incubation, abundant black sclerotia developed on the bean stem tissue.

(ii) Culturing

On PDA, the mycelia of *M. phaseolina* were initially pale white and later turned deep grey. After 36 hours of incubation, the mycelia disintegrated leaving behind black sclerotia (Plate 1).

4:1:2 Slide culture

The mycelia was hyaline and septate. Sclerotia were deep black, widely varied in shape, size and appeared to arise from inter-twinning of the hyphae. The size of the sclerotia ranged between 70-250µm in diameter (Plate 2).

4:1:3 Pycnidia Production

Pycnidia were globose, flask-shaped ranging from 100-250µm in diameter. Conidia were brown, single-celled, fusiform and measured 10-30 x 5-10µ m (Plate 3).

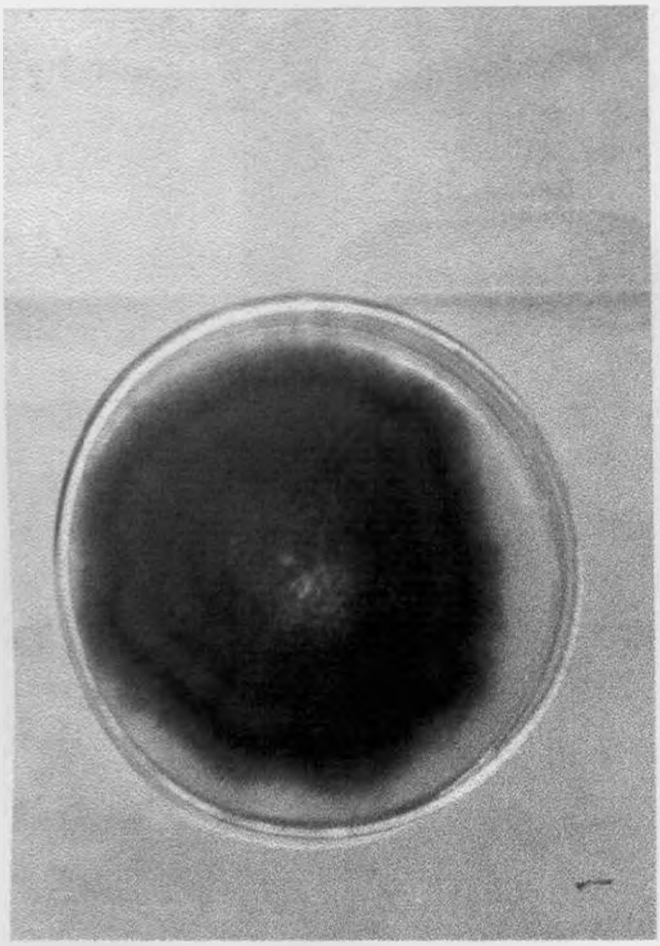


Plate 1 Thirty six (36) hour old culture of *M. phaseolina* growing on PDA

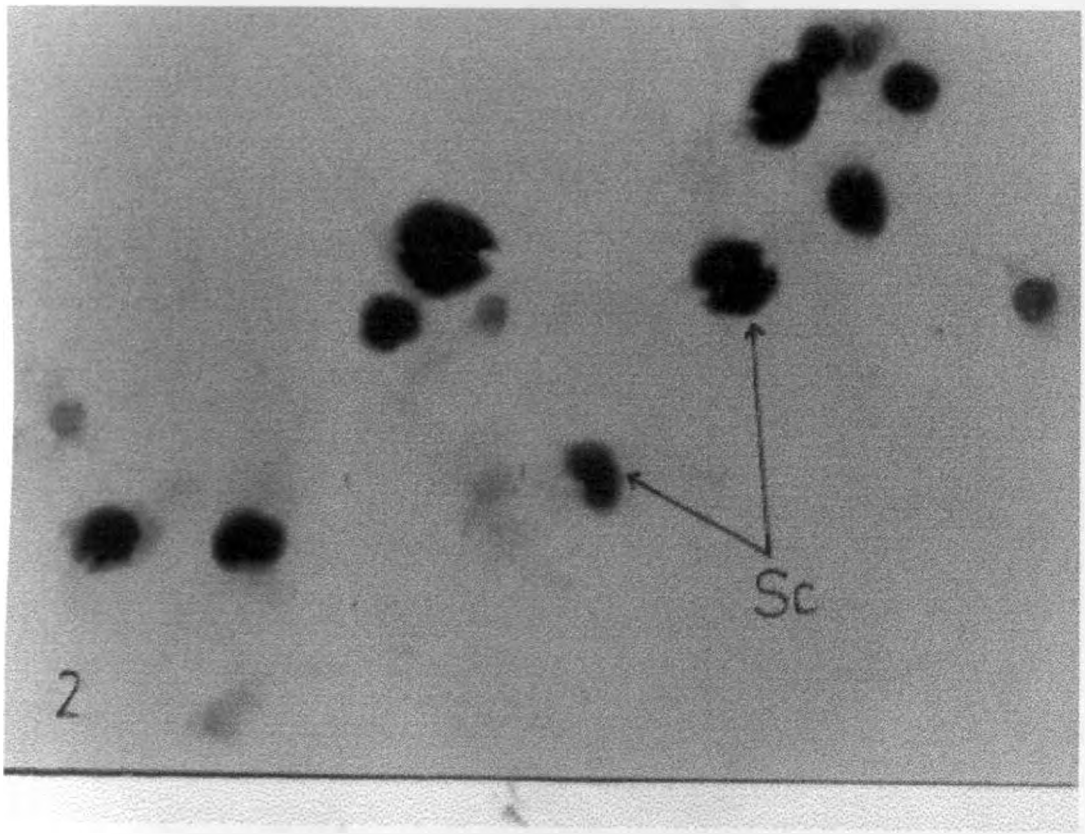


Plate 2. Sclerotia of *M. phaseolina* (x275)

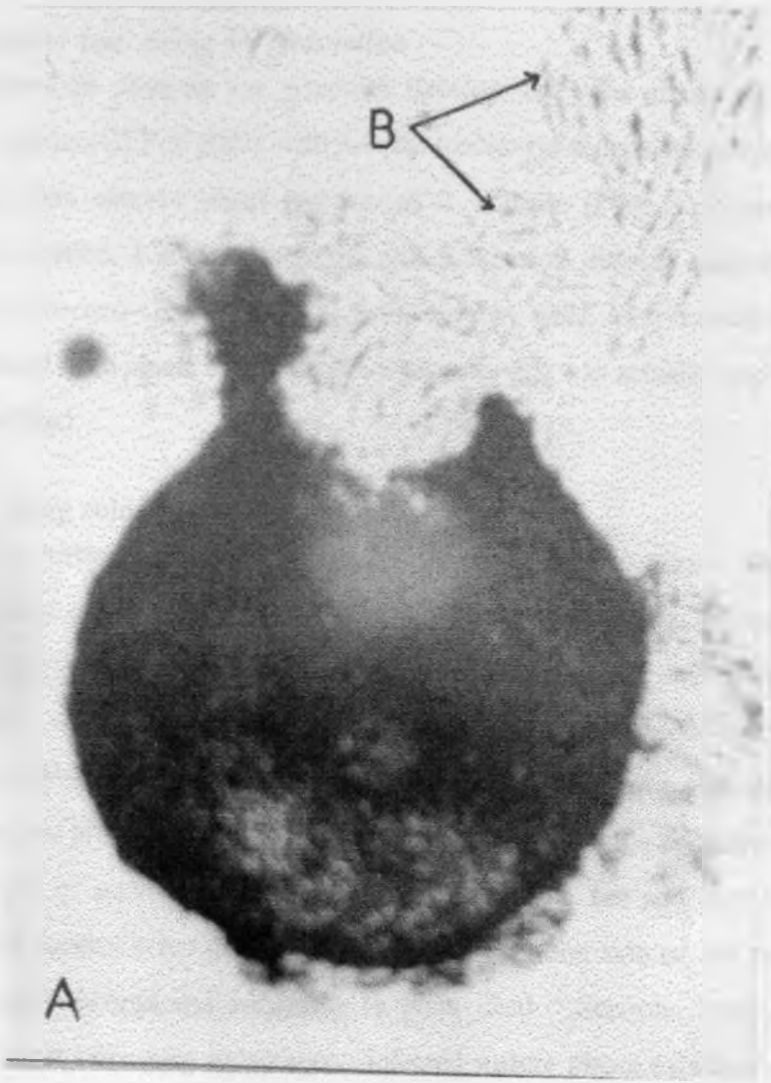


Plate 3. Pycnidia of *M. phaseolina* (A) with pycnidiospores (B) (Magnification x 400).

4:1:4 Pathogenicity test using *M. phaseolina*

Observations on charcoal rot symptom development were made on artificially inoculated cv-Rosecoco-GLP-2 plants with *M. phaseolina* on a daily basis upto 21 days after inoculation when infected plants had started to collapse (Plate 4). Isolates of *M. phaseolina* from Kiambu, Kabete, Machakos and Kakamega districts exhibited similar symptoms on cv-Rosecoco-GLP-2. In both pathogenicity tests, *M. phaseolina* was re-isolated from infected bean plants confirming that the pathogen was actually responsible for the symptoms exhibited.

(i) Inoculation using colonised rice grains

Some seeds virtually decayed due to infection by *M. phaseolina*. Other seeds exhibited pre- and post-emergence damping-off. Post-emergence damping-off was initiated as tiny black lesions at the cotyledonary node and this was observed 5 days after potting. The lesions extended rapidly covering the entire hypocotyl and lower stem, eventually the whole seedling got killed. The dead seedlings developed a characteristic ashy-grey colour on the stems due to the presence of numerous sclerotia embedded in the epidermal layers of stem. In later stages of plant growth, the lesions started from the soil level extending upwards. The black lesions were usually more extensive on one side of the stem. The cankers led to stem breakage and eventually to plant death. Sclerotia were found on infected tissue giving it ashy-grey appearance. Infected mature plants exhibited chlorosis, wilting and did not pod. Developing pods become infected and were covered with sclerotia.

(ii) Innoculation using micro-sclerotia on toothpick

Upon inoculation, black lesions extended through the whole length of the stem causing stem breakage due to the presence of cankers. Black sclerotia were found on the stem giving it a grey appearance.

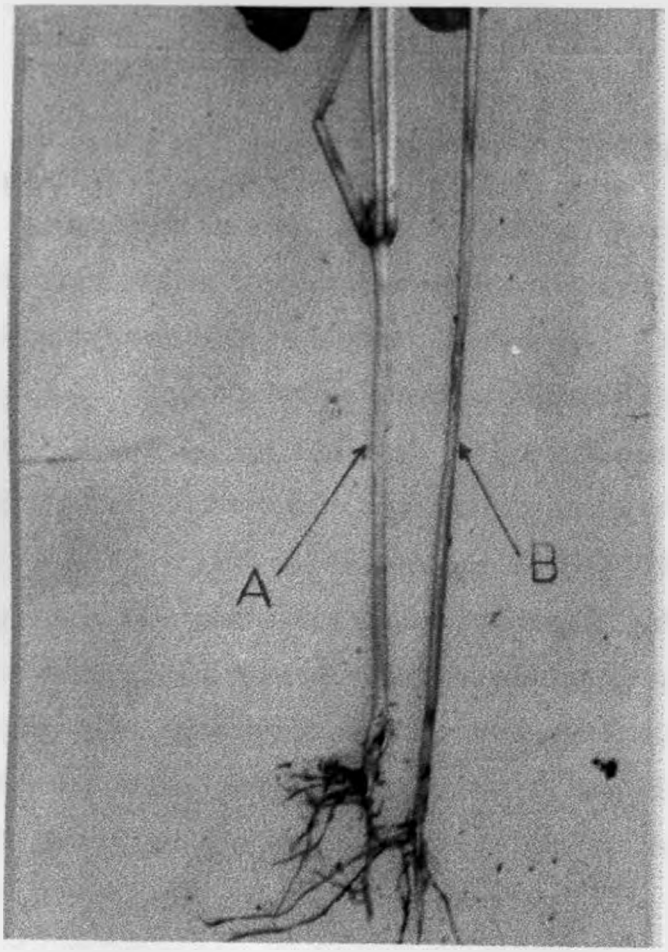


Plate 4. Healthy (A) and infected bean seedlings (B) of cv-Rosecoco GLP-2 inoculated with *M. phaseolina* on rice grains at planting.

4:2:0 In vitro growth of *M. phaseolina*

4:2:1 Effect of growth media, temperature and lighting regimes on mycelial radial growth of *M. phaseolina*

The results on the effect of media type, temperature and lighting regimes on the mycelial radial growth of *M. phaseolina* are shown on Table 2. Results indicate that the mycelial radial growth of *M. phaseolina* is significantly (P=0.05) influenced by media type and temperature but not the lighting conditions (App.2). The mycelial radial growth on MEA and on PDA was not significantly (P=0.05) different from each other but was significantly (P=0.05) higher than the mycelial radial growth on BSDA. The mycelial radial growth was highest at 35°C and this was significantly (P=0.05) higher than the mycelial radial growth at all the other temperatures. There was no significant (P=0.05) difference in the mycelial radial growth recorded at the three different lighting regimes. The interactions between the media type, temperature and light were also significant (P=0.05) in influencing mycelial radial growth. The highest radial growth 63.3mm was recorded on PDA at 35°C under 24 hours of light. The lowest growth of 10.9mm was recorded on BSDA at 22°C at alternate cycles of 12 of light and 12 hours of darkness.

Table 2. Effect of growth media, temperature and light regime on mycelial radial growth (mm) of *M. phaseolina* after 24 hours of incubation.

Media (M)	Incubation Temperature (°c) (T)	Lighting conditions (L) 24D	24L	12L/12D		Mean media (MxT)
(M)						
PDA	(°C)					
	22	40.6	38.3	38.0	30.0	
	25	30.0	41.7	41.0	37.6	
	30	57.0	51.0	51.0	51.3	41.3
	35	58.7	63.3	62.0	61.7	
	40	21.3	27.0	29.0	25.8	
Mean (MXL)		**41.6**	**44.3**	**44.2**		
MEA	22	33.3	33.3	35.1	33.9	
	25	38.7	38.3	38.0	38.3	
	30	57.3	58.0	55.5	57.5	42.0
	35	61.7	60.3	62.0	61.3	
	40	21.7	23.0	20.3	21.7	
Mean (MXL)		**42.5**	**42.5**	**42.2**		
BSDA	22	17.0	15.7	10.9	14.5	
	25	20.3	19.7	18.0	19.3	
	30	23.0	21.0	19.7	18.4	18.4
	35	22.3	21.0	20.0	21.1	
	40	16.7	16.7	15.0	16.1	
Mean (MXL)		**19.9**	**18.8**	**17.9**		
Mean (LXT)					(T)	
	22	32.7	30.8	30.9	31.3	
	25	29.7	31.5	31.4	30.3	
	30	45.8	43.3	44.1	44.4	
	35	46.0	48.2	48.0	47.4	
	40	19.0	22.2	20.7	20.9	
Mean (L)		**34.2**	**35.2**	**35.0**	**34.9**	

	5%	1%
L.S.D.(M)	1.03	1.36
L.S.D.(L)	8.02	9.36
L.S.D.(T)	1.33	1.76
L.S.D.(TxL)	4.00	5.81
L.S.D.(MxT)	4.00	5.81
L.S.D.(MxL)	4.81	7.98
L.S.D.(MxTxL)	3.70	5.06

C.V.(%) = 7.07
S.E. = 1.27

4:2:0 Assessment of *Phaseolus vulgaris* L.cv Rosecoco-GLP-2 seeds from farmers for infection/contamination by *M. phaseolina*

4:2:0 Dry seed examination

4.2.1 Examination of dry sample

Results are shown on Table 3. Significant (P=0.05) differences were revealed among the various seed samples obtained from different areas sampled in each district (App. 3). All the samples from Machakos district had discoloration and malformation associated with *M. phaseolina* infection as sclerotia were readily detected. The sample from Kaitha had an infection level of 10.0% and this was significantly (P=0.05) higher than all the other samples obtained from that district. Only two samples from Kiambu district namely Kamuchege and Ndumberi had discoloration and malformation associated with *M. phaseolina* infection. However samples from various areas did not reveal any significant differences (P=0.05) in the level of infection /contamination. Samples from Kakamega district showed no sclerotia or discoloration that could be attributed to infection by *M. phaseolina*.

Table 3. Percent infection/ contamination by *M. phaseolina* in bean seeds of cv-Rosecoco GLP-2 from Machakos, Kiambu and Kakamega Districts detected by direct examination of dry seeds.

Machakos District		Kiambu District		Kakamega District	
Sample area	% infection	Sample area	% infection	Sample	%infection
Kaitha	10.00a*	Kanjae	0.00 a	Shambalele	0.00
Makutano	6.25b	Mitahato	0.00a	Seremi	0.00
Kivauni	5.50bc	Kia Maiko	0.00 a	Indidi	0.00
Matuu	5.00bc	Kamuchege	0.25 a	Luanda	0.00
Katangi	5.50bc	K.A.R.I. (Vet)	0.00 a	Kakamega Mun.	0.00
Katoloni	3.00cd	K.A.R.I (NARC)	0.00 a	Kaimosi	0.00
Mutituni	1.25de	Tinga'ng'a	0.00 a	Chemoi	0.00
Wamunyu	1.25de	Kanunga	0.00 a	Kapsotik	0.00
Kathekani	1.25de	Ndumberi	0.50 a	Banja	0.00
Mua	6.25b	Gathiga	0.00 a	Butali	0.00
Masii	5.00 bcd	Muthiga	0.00 a	Kabarasi	0.00
Kaani	5.50bc	Njiku	0.00 a	Kiboswa	0.00
*C. S.	0.00f	*C. S.	0.00a	*C. S.	0.00
Mean	4.17	Mean	0.16	Mean	0.00

C.S* certified seeds

*Means followed by similar letters are not significantly different according to Student-Newman-Keul's Test.

4:2:2:0 Non surface sterilised seeds

4:2:2:1 Direct plating of seeds on blotter

With the stereomicroscope or naked eye, black sclerotia around infected seed could be seen. Results are shown in Table 4. The analysis of variance (Appendix 3) to determine the amount of seed contamination/infection revealed significant (P=0.05) differences among the bean seed samples obtained from different areas sampled in each district. In Machakos district, seed sample from Kaitha had the highest infection/contamination level of 6.5% but this was not significantly (P=0.05) higher than the level of infection\contamination found in samples from Makutano and Kivauni. The seed samples from Kaani, Masii, Mua, Kathekani and Wamunyu had levels of infection/contamination that were not significantly (P=0.05) different from each other.

Analysis of variance of seeds from Kiambu plated directly on blotter revealed that samples from Kanjae area had the highest infection/contamination level of 1.75% but this was not significantly (P=0.05) different from samples obtained from Mitahato, Kia Maiko, Kamuchege and K.A. R. I (Vet). The certified seed sample and the sample from Njiku were free from infection.

In Kakamega district the seed sample from Shambalele had the highest infection/contamination level of 0.5% but this was not significantly (P=0.05) different from samples obtained from Seremi, Indidi and Luanda. The seed samples from Kakamega Municipality, Kaimosi, Chemoi, Kapsotik, Banja, Butali, Kabarasi, Kiboswa and the certified seed had no seedborne *M. phaseolina* infection/contamination.

Table 4. Percent infection/ contamination by *M. phaseolina* in bean seeds of cv-Rosecoco GLP-2 from Machakos, Kiambu and Kakamega Districts detected by direct plating on blotter method.

Machakos District		Kiambu District		Kakamega District	
Sample area	% infection	Sample area	% infection	Sample	%infection
Kaitha	6.5a*	Kanjae	1.75a*	Shambalele	0.5a*
Makutano	5.75ab	Mitahato	1.25ab	Seremi	0.25a
Kivauni	4.75ab	Kia Maiko	1.25ab	Indidi	0.25a
Matuu	3.50bc	Kamuchege	1.00abc	Luanda	0.25a
Katangi	3.25bcd	K.A.R.I. (Vet)	1.00abc	Kakamega Mun.	0.00a
Katoloni	3.25bcd	K.A.R.I (NARC)	0.75bc	Kaimosi	0.00a
Mutituni	2.5cd	Tinga'ng'a	0.50bc	Chemoi	0.00a
Wamunyu	2.00cde	Kanunga	0.25bc	Kapsotik	0.00a
Kathekani	1.75cde	Ndumberi	0.25bc	Banja	0.00a
Mua	1.75cde	Gathiga	0.25bc	Butali	0.00a
Masii	1.25de	Muthiga	0.25bc	Kabarasi	0.00a
Kaani	1.25de	Njiku	0.00c	Kiboswa	0.00a
C.S*	0.00e	C.S*	0.00c	C.S*	0.00a
Mean	2.90	Mean	0.66	Mean	0.27

C.S* certified seeds

*Means followed by similar letters are not significantly different according to Student-Newman-Keul's Test.

4.2:2:2 Plating of seeds directly on PDA

With the stereoscopic or naked eye, black sclerotia could be seen around the infected seeds (Plate 5). Results are shown on Table 5. The analysis of variance (Appendix 3) of seed infection revealed significant (P=0.05) differences among the bean samples obtained from different areas sampled in each district.

In Machakos district, seeds plated on PDA without surface sterilization revealed that the seed sample from Kaitha had an infection level of 30.5% that was significantly (P=0.05) higher than that in all the other samples. The seed samples from Masii, Kathekani, Wamunyu, Mutituni and Katoloni had levels of infection/contamination that were not significantly (P=0.05) different from each other. The certified seed had an infection level of 1.5% that was significantly (P=0.05) lower than that in all the other samples.

In Kiambu district, seeds samples from Gathiga had the highest infection level of 4.00% but this was not significantly (P=0.05) different from samples obtained from K.A.R.I. (Vet), Kia Maiko, K.A.R.I. (NARC), Kanjae and Mitahato. The seed samples from Ndumberi, Kanunga, Ting'ang'a, Muthiga, Njiku, Kamuchege and the certified seed had levels of infection/contamination that were not significantly different (P=0.05) among each other.

In Kakamega district, the seed sample from Kiboswa had an infection level of 8.0% that was significantly (P=0.05) higher when compared to all the other samples. The seed samples from Seremi, Luanda, Kakamega Municipality, Kaimosi, Chemoi, Kapsotik, Banja, Butali, Kabarasi, Kiboswa and the certified seed had infection levels that were not significantly (P=0.05) different among each other.

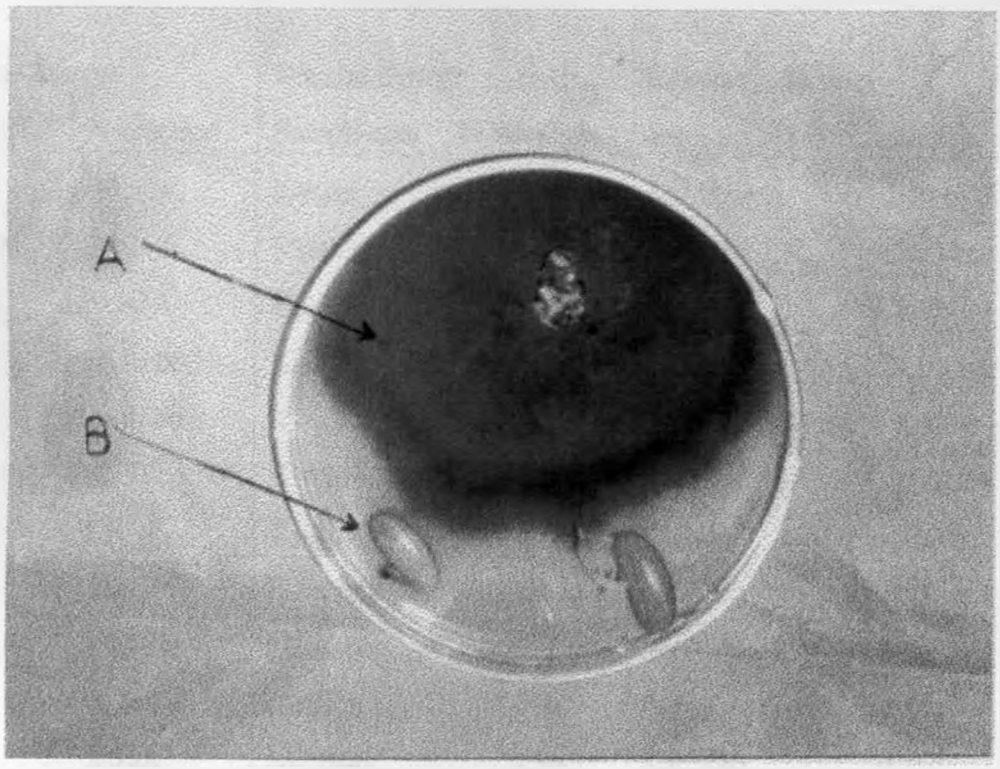


Plate 5. Mycelial (A) growth of *M. phaseolina* emanating from infected seeds on PDA. Healthy seeds (B).

Table 5. Percent infection/ contamination by *M. phaseolina* of bean seeds of cv-Rosecoco GLP-2 from Machakos, Kiambu and Kakamega Districts detected by direct plating on PDA.

Machakos District		Kiambu District		Kakamega District	
Sample area	% infection	Sample area	% infection	Sample	%infection
Kaitha	30.50a	Kanjae	3.25 ab	Shambalele	2.50 b
Makutano	19.25 b	Mitahato	3.00 abc	Seremi	2.00 bc
Kivauni	20.00 b	Kia Maiko	3.50 ab	Indidi	2.5 b
Matuu	15.50 bc	Kamuchege	1.00 d	Luanda	2.00 bc
Katangi	18.50 bc	K.A.R.I. (Vet)	3.75 a	Kakamega Mun.	1.00 bc
Katoloni	12.00 cd	K.A.R.I (NARC)	3.00 abc	Kaimosi	2.00 bc
Mutituni	8.50 d	Tinga'ng'a	1.50 cd	Chemoi	1.00 bc
Wamunyu	8.00 d	Kanunga	1.50 cd	Kapsotik	0.75bc
Kathekani	7.00 de	Ndumberi	2.00 bcd	Banja	1.50 bc
Mua	18.25 bc	Gathiga	4.00 a	Butali	0.00c
Masii	9.00 d	Muthiga	1.25 d	Kabarasi	0.25c
Kaani	17.25 bc	Njiku	1.25d	Kiboswa	8.00 a
C.S*	1.50e	C.S*	1.50cd	C.S*	1.50bc
Mean	14.25	Mean	2.14	Mean	1.92

C.S* certified seeds
*Means followed by similar letters are not significantly different according to Student-Newman-Keul's Test.

4:2:3:0 Plating of surface sterilised seeds

4:2:3:1 Plating of surface sterilised seeds on blotter

With the stereomicroscope or naked eye, black sclerotia around the seed could be seen on infected seeds. Results are shown in Table 6. The analysis of variance (Appendix 3) of seed infection revealed significant (P=0.05) differences among the various seed samples obtained from different districts.

In Machakos district, the seed sample from Makutano and Kaitha had the highest infection level of 3.0% but this were not significantly (P=0.05) different from the infection levels of the sample from Kivauni and Katangi. The seed samples from Kaani, Masii, Mua, Kathekani, Wamunyu and Mutituni had levels of infection\contamination that were not significantly (P=0.05) different among each other.

Analysis of variance of seeds from Kiambu plated after surface sterilisation on blotter revealed that samples from Kanjae area had the highest infection level of 1.5% but this was not significantly (P=0.05) different from infection levels obtained in the sample obtained from Mitahato. The seed samples from Kamuchege, K.A.R.I (Vet), K.A.R.I (NARC), Ting'ang'a, Kanunga, Ndumberi, Gathiga, Muthiga, Njiku and the certified seed had infection levels that were not significantly (P=0.05) different among each other.

In Kakamega district, the seed sample from Kaimosi had the highest infection level of 0.5% but this was not significantly (P=0.05) higher than the levels of infection found in samples from Shambalele, Luanda and Kapsotik. The seed samples from Seremi, Indidi, Kakamega Municipality, Chemoi, Banja, Butali, Kabarasi, Kiboswa and the certified seed had no seedborne *M. phaseolina* infection/contamination.

Table 6. Percent infection/ contamination by *M. phaseolina* of bean seeds of cv-Rosecoco GLP-2 from Machakos. Kiambu and Kakamega Districts detected by plating of surface sterilised seeds on blotter.

Machakos District		Kiambu District		Kakamega District	
Sample area	% infection	Sample area	% infection	Sample	%infection
Kaitha	3.00a	Kanjae	1.50 a	Shambalele	0.25 b
Makutano	3.00 a	Mitahato	1.00 ab	Seremi	0.00 c
Kivauni	2.00 ab	Kia Maiko	0.75 bc	Indidi	0.00 c
Matuu	1.50 bc	Kamuchege	1.00 bcd	Luanda	0.25 b
Katangi	2.00 ab	K.A.R.I. (Vet)	0.50 bcd	Kakamega Mun.	0.00 c
Katoloni	0.75 bcd	K.A.R.I (NARC)	0.00 bcd	Kaimosi	0.50 a
Mutituni	1.00 bcd	Tinga'ng'a	0.00 cd	Chemoi	0.00 c
Wamunyu	1.25 bcd	Kanunga	0.25 d	Kapsotik	0.25 b
Kathekani	0.75 bcd	Ndumberi	0.50 d	Banja	0.00 c
Mua	1.00 bcd	Gathiga	0.50 d	Butali	0.00 c
Masii	0.50 bcd	Muthiga	0.50 d	Kabarasi	0.00 c
Kaani	0.75 bcd	Njiku	0.00 d	Kiboswa	0.00 c
C.S*	0.00d	C.S*	0.00d	C.S*	0.00c
Mean	1.35	Mean	0.39	Mean	0.09

C.S* certified seeds

*Means followed by similar letters are not significantly different according to Student-Newman-Keul's Test.

4:2:3:2 Plating of surface sterilised seeds on PDA

Results are shown on Table 7. The analysis of variance of seed infection revealed significant (P=0.05) differences among the areas sampled in each district (Appendix 3).

In Machakos district, analysis of variance of seeds plated after surface sterilisation revealed that samples from Makutano and Kaitha areas had the highest infection level of 8.00% but the infection level was not significantly (P=0.05) different from that of samples obtained from Kivauni, Katangi and Katoloni whose infection/contamination levels were 6.25%, 5.25% and 4.0% respectively. The seed samples from Mutituni, Wamunyu, Masii and Kaani had infection levels that were not significantly different (P=0.05) among each other.

In Kiambu, the seed sample from Kanjae had the highest infection level of 1.5% but this was not significantly higher (P=0.05) than the infection levels recorded for the seed sample obtained from Mitahato of 1.5%. Seed samples from Kanunga, Ndumberi, Gathiga, Muthiga, Njiku and the certified seed sample showed no seedborne infection caused by *M. phaseolina*.

In Kakamega district, the seed sample from Kiboswa had the highest infection level of 2.0% and this was significantly (P=0.05) higher when compared to all the other seed samples. The seed sample obtained from Luanda had infection level that was significantly (P=0.05) different from all the other seed samples. Seed samples from Indidi, Kakamega Municipality, Chemoi, Kapsotik, Kabarasi and the certified seed showed no seedborne *M. phaseolina*.

Table 7. Percent infection/ contamination of bean seeds by *M. phaseolina* of cv-Rosecoco GLP-2 from Machakos, Kiambu and Kakamega Districts detected by plating of surface sterilized seeds on PDA.

Machakos District		Kiambu District		Kakamega District	
Sample area	% infection	Sample area	% infection	Sample	%infection
Kaitha	8.00a	Kanjae	1.50a	Shambalele	0.25 cd
Makutano	8.00 a	Mitahato	1.00ab	Seremi	0.25 cd
Kivauni	6.25 ab	Kia Maiko	0.75bc	Indidi	0.00 d
Matuu	5.00 bcd	Kamuchege	0.50bcd	Luanda	1.50 b
Katangi	5.25 ab	K.A.R.I. (Vet)	0.50 bcd	Kakamega Mun	0.00 d
Katoloni	4.00 ab	K.A.R.I (NARC)	0.50 bcd	Kaimosi	0.50 c
Mutituni	2.75 de	Tinga'ng'a	0.25 cd	Chemoi	0.00 d
Wamunyu	2.00 ef	Kanunga	0.00 d	Kapsotik	0.00 d
Kathekani	2.50 e	Ndumberi	0.00 d	Banja	0.50 c
Mua	5.00 bc	Gathiga	0.00 d	Butali	0.25 cd
Masii	4.00 bcde	Muthiga	0.00 d	Kabarasi	0.00 d
Kaani	3.00 cde	Njiku	0.00 d	Kiboswa	2.00 a
C.S*	0.00f	C.S*	0.00d	C.S*	0.00d
Mean	4.44	Mean	0.39	Mean	0.38

C.S* certified seeds

*Means followed by similar letters are not significantly different according to Student-Newman-Keul's Test.

4:3:4:0. Grow-on Test

This experiment was set to establish the transmission of *M. phaseolina* from seeds to seedlings under glasshouse conditions and results are shown on Table 8. The analysis of variance (Appendix 3) of seed infection revealed significant (P=0.05) differences among the areas sampled in Machakos and Kiambu districts. Samples from Kakamega district showed no significant (P=0.05) differences among each other.

In Machakos district, the highest transmission of charcoal rot pathogen from bean seeds to seedlings of 10.0% was observed from seedlings raised from bean seeds from Katangi but this was not significantly (P=0.05) different from transmission obtained from seed samples from Kaitha, Kivauni and Makutano. The least transmission was obtained from Katoloni and Kathekani and these seedling infection levels were significantly (P=0.05) lower when compared to other seedling infection.

In Kiambu district, the highest transmission of 8.75% was observed in seedlings raised from bean samples from Kanunga and this was significantly (P=0.05) higher than in all seedlings raised from the samples in that district. There was no disease transmission that was recorded for the samples obtained from Muthiga, Mitahato and the certified seeds.

In Kakamega district, analysis of variance did not reveal significant (P=0.05) differences among various bean samples although the charcoal rot was recorded in bean seedlings raised from all bean seed samples except samples obtained from Indidi, Kakamega Municipality and Chemoi.

Ungerminated seed samples from Kaitha, Kivauni Katangi, Masii, Kanjae, Kamuchege Muthiga and Kiboswa were confirmed to be infected by *M. phaseolina* when plated on PDA.

Table 8. Transmission of *M. phaseolina* from infected/contaminated bean seeds to seedlings detected by grow-on test

Machakos District		Kiambu District		Kakamega District	
Sample area	% infection	Sample area	% infection	Sample	%infection
Kaitha	8.75ab	Kanjae	1.00 c	Shambalele	0.75a
Makutano	8.00 ab	Mitahato	0.00 d	Seremi	0.75 a
Kivauni	8.00 ab	Kia Maiko	0.25 c	Indidi	0.00 a
Matuu	2.50 e	Kamuchege	0.25 c	Luanda	0.50 a
Katangi	10.00 a	K.A.R.I. (Vet)	0.25 c	Kakamega Mun	0.00 a
Katoloni	1.00 f	K.A.R.I (NARC)	0.25 c	Kaimosi	1.25 a
Mutituni	2.50 e	Tinga'ng'a	1.00 c	Chemoi	0.00 a
Wamunyu	2.50 e	Kanunga	8.75 a	Kapsotik	1.75 a
Kathekani	1.25 f	Ndumberi	0.25 c	Banja	0.75 a
Mua	2.75 de	Gathiga	0.50 c	Butali	0.50a
Masii	4.50 c	Muthiga	0.00 d	Kabarasi	0.25 a
Kaani	3.00 d	Njiku	1.50 bc	Kiboswa	1.25 a
C.S*	0.00f	C.S*	0.00d	C.S*	0.00a
Mean	4.21	Mean	1.11	Mean	0.60

C.S* certified seeds

*Means followed by similar letters are not significantly different according to Student-Newman-Keul's Test.

4:2:5:1 Combined analysis of data obtained by various methods

The seed sample obtained from Kaitha had the highest levels of infection as given by direct plating of seed on blotter and on PDA, plating of surface sterilised seeds on blotter and PDA and these were significantly (P=0.05) (App. 3) higher than the level of infection for all the other samples. The seed sample obtained from Katangi had the highest level of infection of 10.0% as determined by the grow-on test and this was not significantly (P=0.05) higher than the level of infection for the samples from Kanunga, Kaitha, Kaani and Kivauni. The seed samples obtained from Mitahato, Muthiga, Indidi, Kakamega Municipality, Chemoi and the certified seed had no seedling infection as detected by the grow-on test. There was no seed borne contamination/infection of *M. phaseolina* detected by all methods on the seed sample obtained from Kakamega Municipality. The seed sample obtained from Kaitha had the highest level of infection of 10.00% as given by the dry seed examination and this was significantly (P=0.05) higher than the level of infection for all the other samples (Table 9). Using unsterilised bean seeds and plating on PDA, the certified seeds PDA showed infection level of 1.5%.

Table 9: The levels of infection/contamination of seeds by *M. phaseolina* as determined by various methods

Sampling area	Seeds plated on blotter		Seeds plated on PDA		Grow-on	dry
	Unsterilised	Surface sterilised	Unsterilised	Surface sterilised	test	seed test
Ting'ang'a	0.25e	0.00d	1.50e	0.00e	1.00d	0.00e
Njiku	0.25e	0.00d	2.00e	0.00e	1.50d	0.00e
Ndumberi	1.00de	0.50cd	1.00e	0.50e	0.75d	0.50e
Kanunga	0.50e	0.25d	1.50e	8.00a	8.75ab	0.00e
Kamuchege	1.25de	1.00c	3.00d	1.00de	0.25de	0.25e
Kanjae	1.75cd	1.50b	4.25d	1.50d	1.00cd	0.00e
Mitahato	0.00e	0.00d	1.25e	0.00e	0.00e	0.00e
Kia Maiko	1.25de	0.75cd	4.50d	0.75de	0.25e	0.00e
Gathiga	0.75e	0.50cd	4.00d	0.75de	0.50e	0.00e
Muthiga	1.00de	0.50cd	3.75d	0.75de	0.00e	0.00e
KARI I(VET)	0.25e	0.50cd	1.25e	0.00e	0.25e	0.00e
KARI II(NARC)	0.25e	0.00d	1.25e	0.00e	0.25e	0.00e
Seremi	0.25e	0.00d	0.75e	0.00e	0.75d	0.00e
Shambalele	0.75de	0.25d	2.00e	0.25de	0.75d	0.00e
Kaimosi	0.75de	0.50cd	2.50e	0.25de	1.25cd	0.00e
Banja	0.25e	0.00d	2.00e	0.50e	0.75d	0.00e
Kapsotik	0.50e	0.25d	3.00d	0.00e	1.75c	0.00e
Luanda	0.75de	0.25d	2.00e	1.50d	0.50d	0.00e
Indidi	0.00e	0.00d	1.00e	0.00e	0.00e	0.00e
Butali	0.00e	0.00d	0.25e	0.25de	0.50d	0.00e
Kabarasi	0.25e	0.00d	1.00e	0.00e	0.25de	0.00e
Kiboswa	0.00e	0.00d	8.00cd	2.00d	1.25cd	0.00e
Kakamega M.	0.00e	0.00d	0.00e	0.00e	0.00e	0.00e
Chemoi	0.00e	0.00d	0.50e	0.00e	0.00e	0.00e
Wamunyu	2.00cd	1.25bc	8.00cd	2.00d	2.50c	1.25d
Kathekani	1.50cd	0.75c	7.00cd	2.50d	1.25cd	1.25d
Mutituni	2.50c	1.00c	8.50cd	2.75cd	2.50c	1.25d
Kaitha	6.50a	3.00a	30.50a	8.00a	8.75ab	10.00a
Matuu	3.50b	1.50b	15.50bc	5.00bc	2.50c	5.00bc
Mua	1.75cd	1.00c	18.50b	5.00bc	2.75c	6.25b
Makutano	5.75a	1.00c	19.25b	8.75a	8.00ab	6.25b
Kaani	1.25de	0.75c	17.25b	3.00cd	3.00cd	5.50bc
Katangi	4.75ab	2.00b	20.00b	6.25ab	10.00a	5.50bc
Masii	3.25b	0.50cd	18.50b	5.25ab	4.50c	5.00cd
Katoloni	1.25de	0.75cd	9.00cd	4.00cd	1.00d	3.00cd
Kivauni	2.00cd	2.00b	12.00bc	6.25ab	8.00ab	3.75bc
CS*	0.00f	0.00d	1.50e	0.00e	0.00e	0.00e
Mean	1.27	0.26	6.06	5.32	6.95	1.52

C.S* certified seeds

*Means followed by similar letters are not significantly different according to Student-Newman-Keul's Test.

4:3:0 Evaluation of Ideal seed health Testing Technique for infection/ contamination by *M. phaseolina*

4:3:1 Correlation coefficients of seed health testing procedure

The correlation coefficients are shown on Table 10. There were positive and significant (P=0.05) correlation coefficients between the dry seed examination and the incubation methods but non-significant correlation with the grow-on test. There was positive and significant (P=0.05) correlation coeficients among the incubation tests. However correlation coefficients between the incubation tests and the grow-on test were not significant (P=0.05).

Table 10. Correlation coefficients for different seed health testing method

Method of seed health testing	1	2	3	4	5	6
1. Dry seed examination						
2. Direct plating on blotter	0.33*					
3. Surface sterilised seeds on blotter	0.72*	0.45*				
4. Direct plating on PDA	0.71*	0.71*	0.86*			
5. Surface sterilised seeds on PDA	0.34*	0.67*	0.78*	0.66*		
6. Grow-on Test	0.04ns	0.15ns	0.26ns	0.23ns	0.21ns	

* Significant correlation coefficients
ns non- significant correlation coefficients (P=0.05)
n=37 df=35
key
1. Dry seed examination
2. Direct plating on blotter
3. Surface sterilised seeds on blotter
4. Direct plating on PDA
5. Surface sterilised seeds on PDA
6. Grow-on Test

4:4:1:0 FIELD EXPERIMENTS

4:4:1 Relationship between seed contamination/infection by *Macrophomina phaseolina* and charcoal rot incidence and severity under field conditions.

4.4.1. Percentage Germination

The prevailing ambient temperatures and rainfall during the field experiments are shown on App 23. Results on seed germination are shown on Table 11. At Kiboko, during the long rains the plots sown with the seed samples having different infection levels had significantly (P=0.05) (App. 4a) different germination percentages. The plots sown with the seed sample having 6.5% infection level had the lowest germination percentage of 80.5 and this was significantly (P=0.05) lower than the germination percentages recorded in all the other plots. The plots sown with the certified seed sample had the highest germination percentage of 91.9 and this was not significantly (P=0.05) different from germination in plots sown with the seed samples having 1%.

At Kiboko during the short rains, the plots sown with the seed sample having 6.5% infection had the lowest germination percentage of 76.0 and this was not significantly (P=0.05) lower than the germination percentage recorded in plots that were sown with the seed sample having 4.75% infection but was significantly lower than the germination recorded in all the other plots. The germination percentages recorded in plots sown with the seed sample having 1%, the hand sorted, the hand sorted surface sterilised and the certified sample were not significantly different (App. 4b) among each other.

At Kabete, for both seasons the germination percentage were not significantly different among plots sown with seed samples having different infection levels (App. 5a and 5b). During the long rains, the plots sown with the seed sample having 6.5% infection level had the lowest germination percentage of 80.7 and this was not significantly (P=0.05) lower than the germination percentages recorded in all the other plots. The plots sown with the seed sample that had 1.0% infection had the highest germination percentage of 89.9 and this was not significantly (P=0.05) different from germination recorded all the other plots (App. 5a).

During the short rains, the plots sown with the seed sample having 6.5% infection

had the lowest germination percentage of 82.5 and this was not significantly (P=0.05) lower than the germination percentage recorded in plots all the other plots (App 5b).

The mean percentage germination for all plots sown with seed samples having different infection levels of 88.6 at Kabete was significantly (P=0.05) higher than the mean percentage germination of 82.3 recorded at Kiboko (App. 5c).

The percentage germination recorded for all plots sown with the seed samples having different infection levels during the short rains of 78.6 was not significantly (P=0.05) (App. 5c) different from the germination percentage of 87.7 recorded in the long rains (Table 11).

The interaction between seed infection level and site was not significant (P=0.05). The interaction between seed infection level and season was also not significant (P=0.05). The interaction between site and season was significant (P=0.05). The interaction between seed infection level, site and season was not significant (P=0.05) (App. 5c).

Table 11. The effect of site, season and different level of seed infection/ contamination by *M. phaseolina* on percentage germination of cv-Rosecoco-GLP-2 seeds.

Season (SE) Site (SI)	long rains	short rains	mean (SI)
Site A (Kiboko)			
Seed infection level (T)			
1	90.1	86.1	
2	86.4	77.6	
3	80.5	76.0	82.3
4	85.2	84.8	
5	86.6	87.4	
6	91.9	90.3	
l..s.d (0.05)	4.40	2.04	
l..s.d (0.01)	6.42	4.09	
Mean (TXSE)	85.8	83.7	
Site B (Kabete)			
1	89.9	95.4	
2	84.8	94.8	
3	80.7	82.5	88.6
4	85.8	88.9	
5	85.7	93.8	
6	85.4	94.8	
l..s.d (0.05)	9.8	14.5	
l. s.d (0.01)	11.0	15.2	
Mean (TXSE)	73.4	91.7	
Mean (SE)	78.6	87.7	
Mean (SIXSE)			
1	90.0	88.0	
2	85.6	81.2	
3	80.6	79.3	
4	85.5	88.9	
5	86.2	90.6	
6	91.5	92.3	
Mean SIxSE	85.6	86.7	

	(0.01)	(0.05)
L.S.D(SI)	1.58	1.19
L.S.D(SE)	12.3	11.9
L.S.D(TxSE)	8.35	5.33
L.S.D(SEXSI)	131.85	26.31

Key

1. Plots sown with seed sample having 1.00% infection as detected by the direct plating on blotter method
2. Plots sown with seed sample having 4.75% infection
3. Plots sown with seed sample having 6.5% infection
4. Plots sown with 6.5% which has been subjected to handsorting
5. Plots sown with 6.5% which has been subjected to handsorting and surface sterilisation
6. Plots sown with certified seeds

4:4:2 The percentage post- emergence damping-off due to charcoal rot

At Kiboko during the long rains, the plots sown with the seed samples having different infection levels had significantly (P=0.05) (App. 6a) different post-emergence damping-off percentages (Table 12). The plots sown with the seed sample having 6.5% infection level had the highest level of post- emergence damping-off of 14.0 % and this was significantly (P=0.05) higher than the level of post- emergence damping-off recorded in all the other plots. The lowest level of post-emergence damping-off of 0.8% was recorded in the plots that were sown with the certified seed sample and this was not significantly (P=0.05) lower than the level of post- emergence damping-off recorded in the plots sown with the hand sorted seed sample of 2.8%. The plots sown with the seed samples having infection level of 1.00%, 4.75% and the hand sorted sample that was surface sterilised had levels of post- emergence damping-off of 6.3, 8.0 and 3.5 respectively and these were not significantly (P=0.05) different among each other.

During the short rains, the plots sown with the seed samples having different infection levels had significantly (P=0.05) (App. 6b) different post-emergence damping-off percentages (Table 12). The plots sown with the seed sample having 6.5% infection level had the highest level of post- emergence damping-off of 8.9% and this was significantly (P=0.05) higher than the levels of post- emergence damping-off recorded in all the other plots. The lowest level of post-emergence damping-off of 0.6% was recorded in the plots that were sown with the certified seed sample and this was not significantly (P=0.05) different from the levels of post- emergence damping-off recorded in the plots sown with the hand sorted seed sample, hand sorted surface sterilised levels and the seed sample having infection level of 1.0%.

At Kabete during the long rains, the post-emergence damping-off percentages recorded in plots sown with the seed samples having different infection levels was not significantly (P=0.05) different among each other (App. 7a). The post-emergence damping-off recorded in plots sown with the seed samples having different infection levels were significantly (P=0.05) different during the short rains. The plots sown with the seed sample having 6.5% infection level had the highest level of post

emergence damping-off of 5.8 % but this was significantly (P=0.05) higher than the levels of post- emergence damping-off recorded in all the other plots. The lowest level of post- emergence damping-off of 0.5% was recorded in the plots that were sown with the certified seed and this was not significantly (P=0.05) lower than what was recorded in all the other the plots (App. 7b).

The mean percentage post- emergence damping-off of 4.7 recorded in the plots at Kiboko was not significantly (P=0.05) different from the mean percentage post- emergence damping-off of 1.7 recorded in the plots at Kabete (App. 7c).

The mean percentage post- emergence damping-off of 3.3 recorded during the long rains season was significantly (P=0.05) (App. 7c) lower than mean percentage post- emergence damping-off of 3.6 recorded in the short rains season (Table 12).

The interaction between the seed infection levels and site was not significant (P=0.05). The interaction between the seed infection levels and season was significant (P=0.05). The interaction between the site and season was not significant (P=0.05). The interaction between the seed infection levels, site and season was not significant (P=0.05) (App. 7c).

Table 12. The effect of site, season and different levels of seed infection/ contamination by *M. phaseolina* on percentage post-emergence damping-off bean seedlings cv-Rosecoco GLP-2.

Season (SE)	long rains	short rains	Mean (SI)
Site (SI)			
Site A (Kiboko)			
Seed infection level (T)			
1	6.3	2.4	
2	8.0	4.6	
3	14.0	8.9	4.7
4	2.8	1.2	
5	3.5	3.4	
6	0.8	0.6	
L.s.d (0.05)	5.0	2.61	
L.s.d (0.01)	7.2	4.71	
Mean (TXSE)	5.9	3.5	
Site B (Kabete)			
1	1.9	1.3	
2	1.3	2.3	
3	1.5	5.8	1.7
4	1.0	2.5	
5	0.5	1.7	
6	0.3	0.5	
L.s.d (0.05)	1.17	3.30	
L.s.d (0.01)	3.16	5.01	
Mean (TXSE)	0.9	3.7	
Mean (SE)	3.3	3.6	
Mean (SIxSE)			
1	3.7	1.9	
2	4.7	3.5	
3	7.8	7.4	
4	1.9	1.9	
5	2.0	2.6	
6	0.6	0.6	
Mean SIxSE	3.5	3.0	
	(0.01)	(0.05)	
L.S.D(SE)	0.13	0.10	
L.S.D(SI)	7.7	5.8	
L.S.D(TxSE)	4.05	2.58	
L.S.D(SEXSI)	64.00	12.77	

Key

1. Plots sown with seed sample having 1.00% infection as detected by the direct plating on blotter method
2. Plots sown with seed sample having 4.75% infection
3. Plots sown with seed sample having 6.5% infection
4. Plots sown with 6.5% which has been subjected to handsorting
5. Plots sown with 6.5% which has been subjected to handsorting and surface sterilisation
6. Plots sown with certified seeds

4:4:3 Incidence of charcoal rot at growth stage V3

At Kiboko, during the long rains, the plots sown with the seed samples having different infection levels had significantly (P=0.05) (App. 8a) different levels of charcoal rot incidence at V3 (Table 13). The plots sown with the seed sample having 6.5% infection level had the highest level of charcoal rot incidence of 23.6% at V3 and this was significantly (P=0.05) higher than the level of charcoal rot incidences recorded in all the other plots. The lowest level of charcoal rot incidence of 3.8% at V3 was recorded in the plots that were sown with the certified seed sample and this was significantly (P=0.05) lower when compared to the levels of charcoal rot incidences at V3 all in the other plots. The plots sown with the seed samples having infection level of 1.00%, hand sorted and the hand sorted surface sterilised samples had levels of charcoal rot incidences at V3 of 9.9%, 6.4% and 4.6% and these were significantly (P=0.05) different from each other.

During the short rains, the plots sown with the seed samples having different infection levels had significantly (P=0.05) (App. 8b) different levels of charcoal rot incidences at V3 (Table 13). The plots sown with the seed sample having 6.5% infection level had the highest level of charcoal rot incidence of 25.9% at V3 and this was significantly (P=0.05) higher than the levels of charcoal rot incidences recorded in all the other plots. The lowest level of charcoal rot incidence of 4.0% at V3 was recorded in the plots that were sown with the certified seed sample and this was not significantly (P=0.05) lower when compared to the levels of charcoal rot incidences at V3 in the plots that sown with the hand sorted surface sterilised sample but was significantly higher than the incidences recorded all in the other plots (App. 8b). The plots sown with the seed samples having infection level of 1.00% and the hand sorted had levels of charcoal rot incidences at V3 that were not significantly (P=0.05) different from each other.

At Kabete during the long and the short rains, the charcoal rot incidences at V3 recorded for plots sown with the seed samples having different infection levels were not significantly (P=0.05) (App. 9a and 9b) different.

During the long rains, the plots sown with the seed sample having 6.5% infection level had the highest level of charcoal rot incidence of 3.2% at V3 and this was not significantly (P=0.05) higher than the levels of charcoal rot incidences recorded in all the other plots sown with the other seed samples. The lowest level of charcoal rot incidence of 1.8 % at V3 was recorded in the plots that were sown with the certified seed but this was not significantly (P=0.05) lower than the incidences recorded in all the other plots sown with the other seed samples (App. 9a).

During the short rains, the plots sown with the seed sample having 6.5% infection level had the highest level of charcoal rot incidence of 3.6% at V3 and this was not significantly (P=0.05) higher than the level of charcoal rot incidences recorded in all the other plots sown with the other seed samples. The lowest level of charcoal rot incidence of 2.7 % at V3 was recorded in the plots that were sown with the certified seed but this was not significantly (P=0.05) lower than what was recorded in all the other plots sown with the other seed samples (App. 9b).

The mean charcoal rot incidence of 10.6% at V3 recorded in the plots at Kiboko was significantly (P=0.05) higher than the mean charcoal rot incidence of 2.8% recorded in the plots at Kabete.

The mean the charcoal rot incidence of 9.0% at V3 recorded during the short rains season was significantly higher (P=0.05) (App. 9c) than the mean charcoal rot incidence of 6.4% recorded in the long rains season (Table 13).

The interaction between the seed infection levels and site was significant (P=0.05). The interaction between the seed infection levels and season was significant (P=0.05). The interaction between the site and season was not significant (P=0.05). The interaction between the seed infection levels, site and season was not significant (P=0.05) (App. 9c).

Table 13. The effect of site, season and different levels of seed infection/ contamination by by *M. phaseolina* on percentage incidence of charcoal rot on beans cv- Rosecoco GLP-2 at growth stage V3.

Season (SE)	long rains	short rains	Mean (S)
Site (SI)			
Site A (Kiboko)			
Seed infection level (T)			
1	9.9	7.4	
2	12.9	15.1	
3	23.6	25.9	10.6
4	6.4	7.8	
5	4.6	4.9	
6	3.8	4.0	
l.s.d (0.05)	0.55	2.20	
l..s.d (0.01)	0.72	4.27	
Mean (TXSE)	10.2	10.9	
Site B (Kabete)			
1	2.5	2.8	
2	2.8	3.2	
3	3.2	3.6	2.8
4	2.6	2.9	
5	2.4	2.8	
6	1.8	2.7	
l s.d (0.05)	1.6	1.2	
l s.d (0.01)	1.9	1.4	
Mean (TXSE)	2.6	7.0	
Mean (SE)	6.4	9.0	
Mean (SIxSE)			
1	6.2	5.1	
2	7.9	9.2	
3	13.4	14.8	
4	3.6	5.9	
5	3.4	3.9	
6	2.8	2.4	
Mean SIxSE	6.2	8.9	
	(0.01)	(0.05)	
L.S.D (SE)	1.33	1.00	
L.S.D (SI)	2.70	3.5	
L.SD (TxSE)	4.05	2.58	
L.S.D (SEXSI)	64 .0	12.78	

Key

1. Plots sown with seed sample having 1.00% infection as detected by the direct plating on blotter method
2. Plots sown with seed sample having 4.75% infection
3. Plots sown with seed sample having 6.5% infection
4. Plots sown with 6.5% which has been subjected to handsorting
5. Plots sown with 6.5% which has been subjected to handsorting and surface sterilisation
6. Plots sown with certified seeds

4:4:4 Incidence of charcoal rot at growth stage R8

At Kiboko, during the long rains, the plots sown with the seed samples having different infection levels had significantly (P=0.05) (App. 10a) different charcoal rot incidences at R8. The plots sown with the seed sample having 6.5% infection level had the highest level of charcoal rot incidence of 40.1% at R8 and this was significantly (P=0.05) higher than the levels of charcoal rot incidences at R8 recorded in all the other plots. The lowest level of charcoal rot incidence of 19.6% at R8 was recorded in the plots that were sown with the certified seed sample and this was not significantly (P=0.05) lower than the levels of charcoal rot incidences at R8 recorded in the plots sown with the seed sample that was hand sorted and then surface sterilised. The plots sown with the seed samples having infection level of 4.75% and 1.00% and hand sorted had levels of charcoal rot incidences at R8 that were not significantly (P=0.05) different among each other but were significantly (P=0.05) different from all the other plots (Table 14).

During the short rains, the plots sown with the seed sample having 6.5% infection level had the highest level of charcoal rot incidence of 62.5% at R8 and this was significantly (P=0.05) (App. 10b) higher than the levels of charcoal rot incidences at R8 recorded of in all the other plots. The lowest level of charcoal rot incidence at R8 was recorded in the plots that were sown with the certified seed sample and this was not significantly (P=0.05) lower than the levels of charcoal rot incidences at R8 recorded in the plots sown with the hand sorted surface sterilised seed sample.

At Kabete during the long rains, the charcoal rot incidences at R8 recorded in plots sown with the seed samples having different infection levels were not significantly (P=0.05) different (App. 11a). During the short rains, the charcoal rot incidences at R8 recorded in plots sown with the seed samples having different infection levels was significantly (P=0.05) different (App. 11b). The plots sown with the seed sample having 6.5% infection level had the highest level of charcoal rot incidence of 7.0% at R8 and this was not significantly (P=0.05) (App. 11b) higher than the level of charcoal rot incidence of 4.0% at R8 recorded in plots that was sown with the seed sample having 4.75% infection but was significantly higher (P=0.05) than the charcoal rot incidence that was recorded in the plots that were sown with the certified seed

sample. However, this incidence level was not significantly (P=0.05) different from charcoal rot incidence that was recorded in plots sown with the hand sorted and hand sorted surface sterilised seed samples

The mean percentage charcoal rot incidence 33.6% at R8 recorded in the plots at Kiboko was significantly (P=0.05) higher than mean percentage charcoal rot incidence of 4.5% at R8 recorded in the plots at Kabete (App.11c).

The mean percentage charcoal rot incidence of 17.7% at R8 recorded during the short rains season was significantly (P=0.05) higher than mean percentage charcoal rot incidence of 15.5% at R8 recorded in the long rains season (Table 14).

The interaction between the seed infection levels and site was significant (P=0.05). The interaction between the seed infection levels and season was significant (P=0.05). The interaction between the site and season was not significant (P=0.05). The interaction between the seed infection levels, site and season was not significant (P=0.05) (App. 11c).

Table 14 The effect of site, season and level of seed contamination/infection by *M.phaseolina* on percent incidence of charcoal rot on bean plants of cultivar Rosecoco GLP-2 taken at growth stage R8

Season (SE)	long rains	short rains	Mean (SI)
Site (SI)			
Site A (Kiboko)			
Seed infection level (T)			
1	28.8	34.5	
2	29.3	47.3	
3	40.1	62.5	33.6
4	30.1	18.8	
5	20.5	13.8	
6	19.6	7.8	
l.s.d (0.05)	7.76	7.72	
l.s.d (0.01)	10.24	9 92	
Mean (TXSE)	26.4	30.8	
Site B (Kabete)			
1	4.4	4.8	
2	4.8	4.0	
3	8.0	7.0	4.5
4	4.0	3.3	
5	3.3	3.0	
6	2.5	2.5	
l.s.d (0.05)	6.55	3.52	
l.s.d (0.01)	7.78	3.80	
Mean (TXSE)	4.5	4.5	
Mean (SE)	15.5	17.7	
Mean (SExSI)			
1	16.7	19.7	
2	17.1	25.7	
3	24.1	34.8	
4	17.1	11.1	
5	11.1	8.4	
6	11.1	5.2	
Mean SIxSE	23.9	4.3	
	(0.01)	(0.05)	
L.S.D (SI)	3.92	2.95	
L.S.D (SE)	2.26	1.70	
L.S.D (TxSE)	12.50	7.47	
L.S.D (SEXSI)	184.93	37.07	

Key

1. Plots sown with seed sample having 1.00% infection as detected by the direct plating on blotter method
2. Plots sown with seed sample having 4.75% infection
3. Plots sown with seed sample having 6.5% infection
4. Plots sown with 6.5% which has been subjected to handsorting
5. Plots sown with 6.5% which has been subjected to handsorting and surface sterilisation
6. Plots sown with certified seeds

4 :4 :5 Severity of charcoal rot

The progress of charcoal rot in plots sown with different seed infection levels are shown in Fig. 1 and App. 14. At Kiboko, during the long rains the different severity levels of charcoal rot recorded in plots sown with different seed samples were significantly (P=0.05) (App. 12a) different. The plots that were sown with the seed sample having 6.5% infection maintained the highest level of severity with a peak of 45.7% recorded 8 weeks after sowing and this was significantly (P=0.05) higher than severity levels in all the other plots. The lowest on severity levels were recorded in the plots that were sown with the certified seed sample and this was significantly (P= 0.05) lower than in all the other plots. The severity levels in plots that were sown with the seed samples that were handsorted and surface sterilised and having 1.0% infection were not significantly (P=0.05) different among each other. The time of sampling after sowing had a significant (P=0.05) effect on the severity score. The interaction between the seed infection level and the time of sampling was also significant (P=0.05).

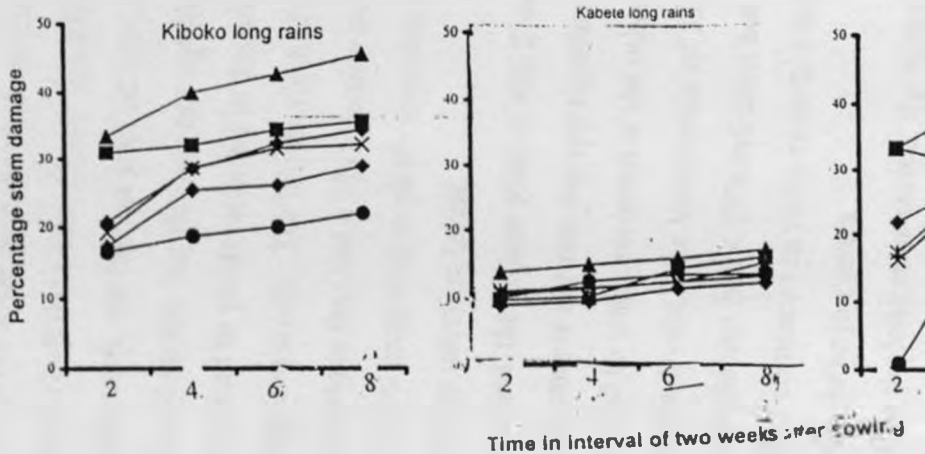
During the short rains, the plots that were sown with the seed sample having 6.5% infection maintained the highest level of severity with a peak of 44.8% recorded 6 weeks after sowing and this was significantly (P=0.05) higher than in all the other plots. The lowest severity levels were recorded in the plots that were sown with the certified seed sample and this was significantly (P= 0.05) lower than in all the other plots. The severity levels in plots that were sown with the seed samples that were handsorted and surface sterilised and having 1.0% infection were not significantly (P=0.05) different among each other. The time of sampling after sowing had a significant (P=0.05) effect on the severity score. The interaction between the seed infection level and the time of sampling was also significant (P=0.05) (App. 12b).

At Kabete, during the long and short rains the severity levels were not significantly (P=0.05) different in plots sown with seed samples having infection levels (App. 13a and b). The severity levels recorded in plots that were sown with the seed sample having 6.5% infection were not significantly (P=0.05) higher than in all the

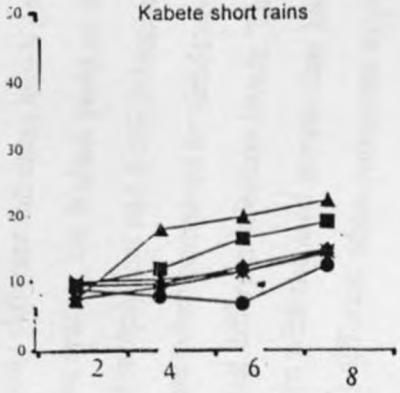
other plots. The lowest severity levels were recorded in the plots that were sown with the certified seed sample and these were not significantly (P= 0.05) lower than in all the other plots. The time of sampling after sowing had a significant (P=0.05) effect on the severity score. The interaction between the seed infection level and the time of sampling was also significant (P=0.05). The mean severity level in the plots at Kiboko was significantly (P=0.05) higher than the mean severity level in the plots at Kabete (App. 13c). The mean severity level during the short rains season was significantly (P=0.05) higher than that during the long rains season.

The interaction between the seed infection levels and site was significant (P=0.05). The interaction between the seed infection levels and season was also significant (P=0.05). The interaction between the site and season was significant (P=0.05). Similarly, the interaction between the seed infection levels, site, season and time of recording was also significant (P=0.05) (App. 13c).

Figure 1 The progress of charcoal rot caused by *Macrophomina phaseolina* in field




Kiboko short rains
4
6
8


Kabete short rains
40
30
20
10
0
2
4
6
8

4:4:6 Yield Parameter

The yield in plots sown with the seed samples having different infection levels was not significantly (P=0.05) (App. 15 and 16) different at both sites and seasons. At Kiboko, during long rains, the highest yield of 1464.4 was recorded in the plots sown with the certified seed sample but this was not significantly (P=0.05) (App 15a) different when compared to yields recorded in the other plots sown with seed samples having different infection levels. The lowest yield of 1182.5 was recorded in the plots sown with the seed sample that had 6.5% infection but this was not significantly (P=0.05) different when compared to yields recorded in the other plots sown with seed samples having different infection levels.

During short rains, the highest yield of 486.2 was recorded in the plots sown with the certified seed sample but this was not significantly (P=0.05) (App. 15b) different when compared to yields recorded in the other plots sown with seed samples having different infection levels. The lowest yield of 316.2 was recorded in the plots sown with the seed sample that had 4.75% infection but this was not significantly (P=0.05) different when compared to yields recorded in the other plots sown with seed samples having different infection levels.

At Kabete during the long rains season, the highest yield of 3319.7 was recorded in the plots sown with the certified seed sample but this was not significantly (P=0.05) different when compared to yields recorded in the other plots sown with seed samples having different infection levels. The lowest yield of 2662.2 was recorded in the plots sown with the seed sample that had 1.0% infection but this was not significantly (P=0.05) different when compared to yields recorded in the other plots sown with seed samples having different infection levels (App 16b)

During the short rains season, the highest yield of 607.8 kg/ha was recorded in the plots sown with the seed sample that had 6.5% infection but this was not significantly (P=0.05) different when compared to yields recorded in the other plots sown with seed samples having different infection levels. The lowest yield of 391.9 was recorded in the plots sown with the seed sample that had 4.75% infection but this was not significantly (P=0.05) different when compared to yields recorded in the other

plots sown with seed samples having different infection levels (App 16b). The mean yield of 856.0 recorded in the plots at Kiboko was significantly (P=0.05) (App. 16c) lower than the yield of 1334.5 recorded in the plots at Kabete. The mean yield of 483.4 recorded during the short rains season was significantly (P=0.05) (App. 16c) lower than mean yield of 1839.1 recorded in the long rains season (Table 15).

The interaction between the seed infection levels and site was significant (P=0.05). The interaction between the seed infection levels and season was also significant (P=0.05). The interaction between the site and season was significant (P=0.05). Similarly the interaction between the seed infection levels, site and season was also significant (P=0.05) (App. 16c)

Table 15. The effect of site, season and level of seed contamination/infection by *M. phaseolina* on yield (kg/ ha) of bean cultivar Rosecoco GLP-2.

Season (SE)	long rains	short rains	Mean (SI)
Site (SI)			
Site A (Kiboko)			
level of seed infection (T)			
1	1293.1	419.1	
2	1353.8	316.2	
3	1182.5	474.1	856.0
4	1235.0	374.4	
5	1306.9	376.4	
6	1464.1	486.2	
Mean (TXSE)	1305.9	407.7	
l.s.d (0.05)	563.1	234.1	
l.s.d (0.01)	652.3	262.3	
Site B (Kabete)			
1	2385.0	594.6	
2	2662.2	391.9	
3	2578.1	607.8	1334.5
4	2854.1	468.0	
5	3001.0	470.5	
6	3319.7	592.6	
Mean (TXSE)	2780.0	592.6	
l.s.d (0.05)	1129.2	282.1	
l.s.d (0.01)	1236.1	563.5	
Mean (SE)	1839.1	483.4	
Mean (SIxSE)			
1	1746.1	506.9	
2	2008.0	354.1	
3	2021.1	547.1	
4	2044.6	421.1	
5	2154.0	423.5	
6	1746.1	533.4	
Mean (SIxSE)	1953.3	464.3	
	(0.01)	(0.05)	
L.S.D (SE)	180	197	
L.S.D (SI)	280	290	
L.S.D (TxSE)	231	230	
L.S.D (SEXSI)	230	130	

Key

1. Plots sown with seed sample having 1.00% infection as detected by the direct plating on blotter method
2. Plots sown with seed sample having 4.75% infection
3. Plots sown with seed sample having 6.5% infection
4. Plots sown with 6.5% which has been subjected to handsorting
5. Plots sown with 6.5% which has been subjected to handsorting and surface sterilisation
6. Plots sown with certified seeds

4:4:9 Percentage yield reduction

The percentage yield reduction in plots sown with the seed samples having different infection levels was not significantly (P=0.05) (App. 17 and 18) different among each other at both sites and seasons. At Kiboko, for both seasons, the highest yield reduction was recorded in the plots sown with the seed sample having high infection level but this was not significantly (P=0.05) (App. 17) different when compared to yield reductions recorded in the other plots sown with seed samples having different infection levels (Table 16).

At Kabete, during the long rains season, the highest yield reduction of 9.63% was recorded in the plots sown with the seed sample that was surface sterilised before sowing but this was not significantly (P=0.05) (App 18) different when compared to all the other plots sown with seed samples having different infection levels. For the short rains season, the highest yield reduction of 13.73% was recorded in the plots sown with the seed having high infection level and this was not significantly (P=0.05) different when compared to the other plots sown with seed samples having different infection levels (App. 18c).

The interaction between the seed infection levels and site was significant (P=0.05). The interaction between the seed infection levels and season was also significant (P=0.05). The interaction between the site and season was significant (P=0.05). Similarly the interaction between the seed infection levels, site and season was also significant (P=0.05) (App. 18c) (Table 16).

Table 16. Percent yield/ha reduction (based on yields from plots sown with certified seed) for plots sown with seeds having varying levels of infection/contamination by *M. phaseolina*

Season	long rains	short rains	Mean (SI)
Site			
Site A (Kiboko)			
level of seed infection (T)			
1	11.47	23.30	
2	14.35	25.00	
3	15.52	26.68	18.45
4	13.92	21.03	
5	12.60	20.60	
Mean (TXSE)	13.52	23.32	
L.S.D (0.05)	5.2	7.2	
L.S.D (0.01)	5.8	8.1	
Site B (Kabete)			
1	7.30	11.01	
2	7.37	8.90	
3	7.96	13.73	9.84
4	8.82	12.96	
5	9.63	10.63	
Mean (TXSE)	8.22	23.32	
Mean (SE)	10.87	17.26	
L.S.D (0.05)	3.7	4.3	
L.S.D (0.01)	4.2	5.5	
Mean (SEXSI)			
1	9.39	17.16	
2	10.9	16.95	
3	11.74	20.21	
4	11.37	17.00	
5	11.12	15.62	
Mean (SEXSI)	10.90	17.39	
	(0.05)	(0.01)	
L.S.D (SE)	4.82	5.10	
L.S.D (SI)	6.21	6.72	
L.S.D (TxSE)	1.31	1.30	
L.S.D (TxSI)	2.30	2.03	
L.S.D (SEXSI)	2.30	1.30	
L.S.D (SEXSIXT)	1.34	1.29	

Key

1. Plots sown with seed sample having 1.00% infection as detected by the direct plating on blotter method
2. Plots sown with seed sample having 4.75% infection
3. Plots sown with seed sample having 6.5% infection
4. Plots sown with 6.5% which has been subjected to handsorting
5. Plots sown with 6.5% which has been subjected to handsorting and surface sterilisation

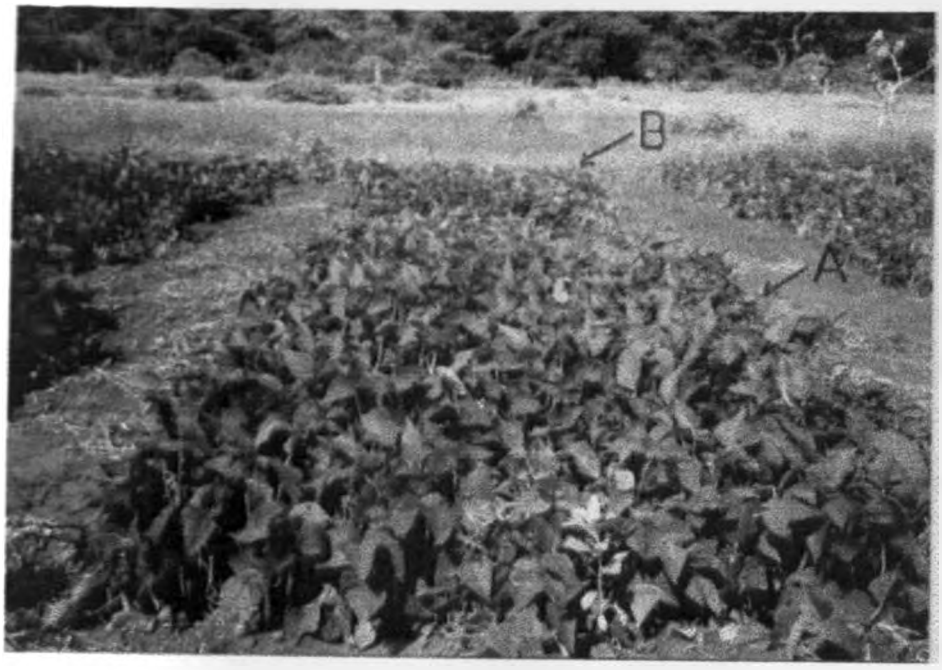


Plate 6. Plots planted with certified bean seeds (A)
seeds with high infection level (B) at Kiboko.

4:4:9 Correlation Matrix

During both the long and short rains at Kiboko, the correlation matrix analysis for yield and severity was negative and non-significant (P=0.05) (Table 17a and 17b). The correlation between seed infection level and germination was negative and significant (P=0.05). There was positive and significant (P=0.05) correlations between seed infection level and charcoal rot incidence at V3 and R8 and severity. The correlation between seed infection level and yield was negative and significant (P=0.05).

At Kabete all the correlations between different parameters were non-significant (P=0.05) during the long rains. During the short rains, there was positive and significant (P=0.05) correlation between seed infection level and incidence at R8 (Table 18a and b) all the other correlations between the other parameters were non-significant.

Table 17. The correlation analysis matrix for various parameters recorded at Kiboko.

(a) Long rains

	germination	post-emergence	V3	R8	severity	yield
Germination						
Post emergence damping-off	0.217ns					
Yield					-0.161ns	
Seed infection	-0.442*	0.112ns	0.566*	0.602*	0.971*	-0.741*

(b) Short rains

	germination	post-emergence	V3	R8	severity	yield
Germination						
Post emergence damping-off	0.113ns					
Yield					-0.221ns	
Seed infection	-0.588*	0.222ns	0.664*	0.722*	0.721*	-0.765*

Ns-non-significant correlations

* significant correlations (P=0.05)

Table 18. The correlation analysis matrix for various parameters recorded at Kabete.

(a) long rains

	germination	post-emergence	V3	R8	severity	yield
Germination						
Post emergence damping-off	0.017ns					
Yield					0.107ns	
Seed infection	0.001ns	0.101ns	0.002ns	0.062ns	0.004ns	0.221ns

(b) short rains

	germination	post-emergence	V3	R8	severity	yield
Germination						
Post emergence damping-off	0.217ns					
Yield					0.261ns	
Seed infection	-0.132ns	0.112ns	0.366ns	0.602*	0.171ns	0.141ns

Ns-non-significant correlations

* significant correlations (P=0.05)

4:5:0 Artificially Inoculated plots

4:5:1 Yield Parameter

Results recorded in plots where inoculations were carried out at different stages of growth are shown on are shown on Table 19. At Kiboko during the long rains season, the yields recorded in plots where inoculations were carried out at different growth stages was not significantly (P=0.05) different among each other (App. 19a). The lowest yields of 120.0 was recorded in the plots that were inoculated 8 weeks after sowing while the highest yields of 253.0 was recorded in plots that were inoculated with sterile tooth picks 2 weeks after sowing.

During the short rains season, the yield recorded in plots where inoculations were carried out at different growth stages were significantly (P=0.05) different each other. The lowest yield of 151.1 was recorded in plots where inoculations were carried out at planting and this was significantly (P=0.05) higher than the yields recorded in all the other plots where inoculations were done at other growth stages. The yields that was recorded in plots where inoculations were carried 2, 4, 6 and 8 after sowing were not significantly (P=0.05) different among each other. The highest yield of 756.8 was recorded in plots where inoculations were carried out two weeks after sowing with sterile tooth picks (App 19b) and this was significantly (P=0.05) higher when compared to yields in all the other plots.

At Kabete for both seasons, the yields recorded in plots where inoculations were carried out at indicated growth stages were not significantly (P=0.05) different among each other (App.20a and 20b). During the long rains, the highest yields of 3320.0 was recorded in plots that were inoculated with sterile tooth picks 2 weeks after sowing while the lowest yields of 1226.2 was recorded in the plots that were inoculated 4 weeks after sowing.

The mean yield of 252.6 recorded in the plots at Kiboko was significantly (P=0.05) (App. 20c) lower than the yield of 2034.0 recorded in the plots at Kabete. The mean yield of 642.4 recorded during the short rains season was significantly (P=0.05) lower than mean yield of 1450.0 recorded in the long rains season (Table 19). The interaction between the growth stage at which the inoculations were carried out

and site was significant (P=0.05). The interaction between the growth stage at which the inoculations were carried out and season was also significant (P=0.05). The interaction between the site and season was significant (P=0.05). Similary the interaction between the growth stage at which the inoculations were carried out, site and season was also significant (P=0.05) (App. 20c).

Table 19. The yield recorded in plots inoculated with *M. phaseolina* at different stages of growth

Season (SE)	long rains	short rains	Mean (SI)
Site (S)			
Site A (Kiboko)			
level of seed infection (T)			
1	220.5	151.1	
2	191.6	241.1	
3	219.6	274.8	252.6
4	243.7	304.6	
5	120.0	239.0	
6	147.8	253.5	
7	253.0	756.8	
Mean (TXSE)	199.5	297.7	
l.s.d(0.05)	157.2	87.9	
l.s.d(0.01)	162.3	93.7	
Site B (Kabete)			
1	2574.0	1180.0	
2	2585.0	910.8	
3	2262.0	1078.8	
4	2578.0	1107.0	2034.5
5	2584.0	778.0	
6	3001.0	871.3	
7	3320.0	982.2	
l.s.d(0.05)	1129.0	562.0	
l.s.d(0.01)	1142.0	585.0	
Mean (TXSE)	2700.0	987.0	
Mean (SE)	1450.0	642.4	
Mean (SIxSE)			
1	1397.3	710.6	
2	1388.2	530.5	
3	1240.8	814.3	
4	1410.9	705.8	
5	1352.0	401.0	
6	1648.2	508.7	
7	1786.5	562.4	
Mean (SIxSE)	1953.3	604.8	
	(0.01)	(0.05)	
L.S.D (T)	1.30	93	
L.S.D (SI)	1.80	197	
L.S.D (SE)	130	154	
L.S.D (TxSE)	230	203	
L.S.D (SEXSI)	230	130	

Key 1. Inoculated at planting
2. inoculated 2 weeks after planting
3. inoculated 4 weeks after planting
4. inoculated 6 weeks after planting
5. inoculated 8 weeks after planting
6. inoculated at physiological maturity i.e. 10 weeks after sowing
7. inoculated with sterile toothpicks 2 weeks after sowing

4:5:2 Relation between time and level of charcoal rot infection of cv-Rosecoco GLP-2 and the resultant seedborne *M. phaseolina* in harvested seeds

The main objective of this experiment was to establish the critical time of charcoal rot infection that would result in detectable charcoal rot infection of harvested seeds.

Results for subsequent seed infection are shown on Table 20. At Kiboko, during long rains, the highest resultant seed infection of 13.0% was recorded in beans seeds from plots where inoculations were carried out at planting was and this was significantly (P=0.05) higher when compared to seed infection/contamination recorded in all the other plots. The resultant seed infection in plots where inoculations were carried 2 weeks and 4 weeks after sowing were not significantly (P=0.05) different from each other (App.21a).

During the short rains, the resultant seed infection of 1.25% in bean seeds from plots where inoculations were carried out at planting was the highest and this was not significantly (P=0.05) higher than the infection level recorded in beans seeds from plots where inoculations were carried 2 weeks, 4 weeks and 6 weeks after sowing. The lowest resultant seed infection 0.14% was recorded in seeds obtained from plots that were inoculated with sterile tooth picks and this was significantly (P=0.05) lower than the infection level recorded in seeds obtained from the plots inoculated at planting (App.21b).

At Kabete for both seasons, the resultant seed infection in plots where inoculations were carried out at various growth stages were not significantly (P=0.05) different among each other (App.22a and 22b). For both long and short rains, the lowest infection levels of 0.13% and 0.07% respectively were recorded in plots that were inoculated with sterile toothpicks two weeks after sowing.

The mean resultant infection level of 5.28% recorded in the plots at Kiboko was significantly (P=0.05) higher than the mean resultant infection level of 0.85 % recorded in the plots at Kabete (App. 22c).

The mean resultant infection level recorded during the short rains season was significantly (P=0.05) lower than the resultant infection level recorded recorded in the long rains season (App. 22c).

The interaction between the growth stage at which the inoculations were carried out and site was significant (P=0.05). The interaction between the growth stage at which the inoculations were carried out and season was also significant (P=0.05). The interaction between the site and season was significant (P=0.05). Similiary the interaction between the growth stage at which the inoculations were carried out, site and season was also significant (P=0.05) (App. 22c).

Table 20. The percentage subsequent infection/contamination recorded for seeds harvested from plots inoculated with *M. phaseolina* at different stages of growth

Season (SE)	long rains	short rains	Mean (SI)
Site (SI)			
Site B (Kiboko)			
1	13.00	1.25	
2	11.25	1.00	
3	11.25	0.50	5.28
4	9.75	0.25	
5	7.75	0.25	
6	4.25	0.17	
7	2.1	0.14	
l.s.d(0.05)	0.67	1.06	
l.s.d(0.01)	0.93	1.47	
Mean (TXSE)	9.54	0.63	
Site A (Kabete)			
Stage of inoculation (T)			
1	2.67	0.67	
2	1.50	0.50	
3	1.17	0.25	0.85
4	1.25	0.33	
5	0.83	0.08	
6	0.33	0.17	
7	0.13	0.07	
l.s.d(0.05)	2.57	0.58	
l.s.d(0.01)	2.79	0.99	
Mean (TXSE)	1.29	0.33	
Mean (SE)	5.7	0.51	
Mean (TxSE)			
1	7.8	0.96	
2	6.4	0.75	
3	6.3	0.63	
4	5.5	0.29	
5	4.3	0.17	
6	2.4	0.17	
7	0.32	0.37	
Mean (SIxSE)	5.5	0.50	
	(0.01)	(0.05)	
L.S.D (T)	1.4	0.94	
L.S.D (SE)	3.2	2.4	
L.S.D (SI)	2.3	1.2	
L.S.D (TxSE)	2.2	2.2	
L.S.D (SEXSI)	8.6	43.2	

Key

1. Inoculated at planting
2. inoculated 2 weeks after planting
3. inoculated 4 weeks after planting
4. inoculated 6 weeks after planting
5. inoculated 8 weeks after planting
6. Inoculated at physiological maturity i.e. 10 weeks after sowing
7. inoculated with sterile toothpicks 2 weeks after sowing

4:5:5 Correlation Matrix

There was positive and significant correlation between seed infection and stage of inoculation at Kiboko but not at Kabete (Table 21 and 22). There was no significant correlation between stage of inoculation and yield

Table 21. The correlation matrix of stage of inoculation, yield and subsequent seed infection at Kiboko

(a) Long rains

	yield	seed infection
Seed infection	0.866*	
Stage of inoculation	0.112ns	0.677*

(b) short rains

	yield	seed infection
Seed infection	0.866*	
Stage of inoculation	0.076ns	0.604*

Table 22. The correlation matrix of stage of inoculation, yield and subsequent seed infection at Kabete.

(a) long rains

	yield	seed infection
Seed infection	0.122ns	
Stage of inoculation	0.052ns	0.07ns

(b) Long rains

	yield	seed infection
Seed infection	0.122ns	
Stage of inoculation	0.015ns	0.11ns

CHAPTER FIVE

5:1:0 DISCUSSION

5:1:1 Cultural and Morphological Characterisation of *Macrophomina phaseolina*

5:1:2 Morphological characterisation of *M. phaseolina*

Morphological characteristics used in this study to identify *M. phaseolina* did conform to those observed by other workers. Mycelia were septate and branched, initially pale white, later turning smoky grey and after 36 hours disintegrating leaving behind black sclerotia which were evenly distributed on the media surface. The sclerotia were diverse in terms of shape and size releasing oil globules when pressure was applied. Coley-Smith and Cooke (1971) reported similar findings. The sizes of the sclerotia ranged between 70-250µm in diameter. Pycnidia ranged between 120-250µm in diameter and pycnospores were hyaline to brown and ranged between 10-25µm x 5-10µm (Alexopoulus and Mims, 1979; Sutton, 1980 and Songa, 1995).

Fungi have an optimum temperature for mycelial radial growth. There is also minimum and maximum temperature below and above which growth will not occur (Roberts and Boothroyd, 1984). Mycelial growth occurred at a wide temperature range but grew best between 30-35°C. This is in agreement with findings by Songa (1995) working on the same pathogen.

M. phaseolina grew rapidly on both MEA and PDA at the optimum temperatures of 30-35°C. Andrus (1938) and Songa (1995) reported good mycelial radial growth on PDA. Poor growth was reported in BSDA. Songa (1995) reported poor growth on rice based medium and attributed this to it's poor nutritional status. This might have also been the case with BSDA or the presence of inhibitory factors in this medium.

Roberts and Boothroyd (1984) reported that in many fungi, mycelial radial growth is influenced by different lighting regimes. However in this work the light regime did not have any effect on the radial growth of *M. phaseolina*. Continous darkness, continous light and alternate cycles of 12 hours of darkness and 12 hours of light all gave good growth. This is not in agreement with Thirumalarchar (1953) who reported that *M. phaseolina* had comparatively better growth under total darkness. The *M. phaseolina* isolates obtained

locally were probably different from those that he was working with and therefore the difference in response towards growth conditions

5:1:2 Assessment of cv-Rosecoco-GLP-2 seeds from farmers for infection/contamination by *M. phaseolina*.

M. phaseolina occurred in samples from all the districts but the incidence of infected samples varied among districts. It was relatively low in samples from Kakamega but high with internally borne infections in Machakos and Kiambu. Seed samples from Kaitha, Kivauni and Katangi in Machakos district had high levels of infection/contamination as these areas are characterised by high temperatures and low rainfall that favour the growth and establishment of *M. phaseolina*. The environmental conditions for the manifestation of a disease in any locality is confirmed (Sattar, 1933). Conditions favouring early infection such as warm temperatures could encourage systemic seed infection and this probably explains why all the seed samples from Machakos district were all infected/ contaminated. The climatic conditions prevailing at Kakamega district are too humid for growth and proliferation of *M. phaseolina* and this explains the low levels of seed infection/contamination by the pathogen. The seed sample obtained from Kakamega Municipality was free of seed infection/contamination by *M. phaseolina*. High soil moisture reduces the survival of *M. phaseolina* in the soil (Dhingra and Sinclair, 1975). Reduction in activity at increased moisture levels has been attributed to increased bacterial activity, which causes lysis of mycelium and inhibition of sclerotia germination (Bhattacharya and Saadar, 1976). Bean plants may be infected during the early growth stages therefore the inoculum establishes deep inside the seed, precisely in the cotyledons. Infected seeds have been harvested from symptomless plants. Seed contamination result from the contact of pods with infested soil. Songa (1995) reported that during threshing, the sclerotia may attach to the seed surface. Kendrick (1933) and Andrus (1938) both reported that internally and externally borne inoculum is capable of causing disease. Spores and other reproductive organs are often carried on the surface of the seed. In this way many smut fungi and seed borne species of *Alternaria* may be transmitted. Neergard (1979) found out that uredospores of *Puccinia antirrhini* adhere to the seed surface and act as a

source of *Antirrhinum majus*. Baker (1952) reported that the aster wilt organism, *Fusarium oxysporum* f. sp. *callistephi* conidia smeared on the seed surface grows into the soil and invades the seedling through the roots and once introduced into the soil the pathogen may persist for many years.

Schonherr and Mbugua (1976) and Rono and Shakoor (1990) reported that in Kenya, most small scale farmers do not use certified seeds but that they obtain seeds from the previous crop or from the local market. The use of such seeds should be discouraged as they might be infected and likely to cause diseases in the field. Buruchara (1979) reported high incidences of Bean Common Mosaic Virus in bean seeds obtained from farmers. Similarly, Origa (1992) and Mwang'ombe *et. al.* (1995) reported high incidences of *Pseudomonas syringae* pv *phaseolicola*, the causal agent of halo blight in bean seeds obtained from farmers. This was further confirmed by Isanda (1995) who reported high incidences of *Colletotrichum lindemuthianum*, the causal organsim of bean anthracnose in farmers seeds. Girsh *et.. al.* (1996) who evaluated the quality of self-saved seed of barley reported that self-saved seed had more incidences of weed seed contamination, seedborne diseases like *Ustilago nuda*, *Tilletia indica* and *Cochliobolus nodorum* than certified seed.

In some of the seed samples, exhumed seeds were reported to be decayed as a result of *M. phaseolina* which causes rotting of the infected seeds. Loss of germination was probably due the activity of the fungus as reported by Andrus (1925) and Abawi-and Pastor-Corrales (1986). Dipawanita *et. al.* (1994) reported that inhibition of seed germination is by a toxin called phaseolinone and the degree of inhibition of seed germination was correlated well with the amount of toxin produced. However other seeds failed to germinate as they were probably infected by other seed borne micro-organisms namely *Fusarium* spp. and *Colletotrichum lindemuthianum* among others.

The seed transmission of *Macrophomina phaseolina* allows spread to previously disease free areas and can introduce strains of the pathogen to any area. The movement of seed from the marginal areas to Kiambu district might have contributed to disease out breaks in the area resulting in seedborne infection. Seed certification programmes normally set up tolerance levels for infected seed with the implication that the economic loss from the ensuing diseased plants will be less than the cost of producing seed free from the pathogen

or of treating seed to free it from the disease agent. This overlooks the importance of introducing the pathogen or a strain into a new area as well as the rapidity with which some pathogens increase from small initial population. The transmission of seedborne pathogens across boundaries cannot be underestimated especially in Sub-Saharan Africa where cross border trading is common through unofficial routes which the governments do not control. These imported seed can introduce seedborne diseases as no inspections are done. Imported seeds are normally treated with chemicals thereby pathogens are not detected at entry points. However, cumulatively with time, new diseases or strains are reported (Neergard, 1979).

5: 1: 3 Ideal Seed health Testing Method

Ideal seed health testing procedure should give reliable information and the results must be reproducible. The dry seed examination gives a general idea about the health status of seed samples as seed infection is manifested as shrivelling and discoloration and can be used during handsorting to remove malformed and discoloured bean seeds. Bean seed samples from Kaitha and Kivauni had an ashy grey appearance due to the presence of numerous sclerotia embedded on the seed surface. Andrus (1938) and Songa (1995) reported similar findings when working with bean seeds infected by *M. phaseolina*. Incubation tests confirmed that these samples were highly infected/ contaminated by *M. phaseolina*. Otieno (1992) reported higher incidences of halo blight caused by *Pseudomonas syringe* p.v. *phaseolicola* on malformed seeds. Isanda (1995) similarly reported that malformed and discoloured seeds had higher incidences of bean anthracnose caused by *Colletotrichum lindemuthianum*. This is an indispensable procedure necessary to supplement incubation procedures but cannot be used independently. Neergard (1979) reported that certain fungi detectable by direct inspection such as *Septoria* on celery may be dead. Weak incidences of fungi may not show external signs therefore suitable incubation procedures based on development of fungi are imperative to provide full information.

Direct plating of seeds on blotter was relatively cheap and quick method of evaluation of seed health. Recording was also quick and identification of characters of *M.*

phaseolina and including features such as form, length, size, septation and sclerotia is easy (Neergard, 1979). Identification of *M. phaseolina* was also straight forward as sclerotia are formed abundantly on the blotter and on the seed surface. This method can be carried out in poorly equipped laboratories where even basic facilities are lacking. This is in agreement by Mathur and Sackston (1963) who recommended direct plating of seeds on blotter for evaluation for seeds for infection by *M. phaseolina*. This method is highly recommended, as it's a hybrid between the moist chamber procedure used in plant pathology and the germination test used in seed technology. It provides the advantages of *in vitro* investigations with those of *in vivo* observations (Neergard, 1979). Direct plating of seeds on PDA was a very sensitive method resulting in higher incidences of *M. phaseolina* in seeds compared to other methods. Umechuruba and Nwachukwu (1995) who compared the efficiency of different seed health testing methods reported similarly that the agar method was very sensitive in detection of *M. phaseolina* in African Yam bean. In this method however there was rapid growth of saprophytic and other pathogenic fungi that could hamper quick identification of the pathogen. Neergard (1979) reported similar findings. Seeds plated without surface sterilisation on blotter and PDA had higher incidences of *M. phaseolina* implying that the inoculum is located on the seed surface and beneath the seed surface. Kendrick (1933), Andrus (1938) and Songa (1995) reported similiar findings in bean seeds. Pre-treatment with a surface sterilant eliminates externally located inoculum therefore does not give a true picture of the inoculum level in the seed and should not be used in seed health testing.

The development of diseased seedlings from bean seeds infected by *M. phaseolina* confirms that the pathogen is seed transmitted and is in agreement with earlier findings by Kendrik (1933).

The positive and significant correlation between the incubation tests imply the applicability of the agar and the blotter tests for the detection of seedborne *M. phaseolina*. Mathur and Sackston (1963) reported similar findings for detection of seed borne *M. phaseolina* in beans. There was also positive and significant correlation between the dry seed inspection and the incubation test. The findings are in agreement with Isanda (1995) who had similar findings when working with bean seeds infected by *C. lindemuthianum*.

The non- significant correlation between dry seed examination, incubation tests and grow-on test implying that the transmission of charcoal rot from seeds to seedlings may be determined by other factors in the soil. For instance highly infected seeds never germinated thus lowering the transmission level to seedlings. Neergard (1979) reported that the composition of soil mycoflora may determine the development of pathogenic fungi introduced in the soil with seed. Competition occurs between the organisms for access to nutritional material and the relationship between organisms may positively be dependent, stimulating as in synergism or hampering as in antagonism. Contaminated seed if sown in disinfested soil may lead to more severe development of diseases than contaminated seed sown in untreated soil where antagonistic soil microorganisms are not eliminated. Neergard (1979) reported that *Drechslera sorokiniana*, which is sensitive to the effect of other fungi, developed vigorously in sterilised soil but could almost be suppressed when a little unsterile soil was added. Manadhar *et. al.* (1998) similarly reported high incidences of *Pyricularia oryzae* in rice grown in sterile soil than seeds grown in unsterilised soil.

5:1:4 The influence of seed infection and contamination on disease incidence and severity in the field

The occurrence of natural seed transmission of charcoal rot is an important aspect in accounting for the disease outbreaks when farmers use their own seed. During the short rains season, both at Kabete and Kiboko, germination percentages in all the plots sown with seeds having different levels of infection was not significantly different. The higher prevailing temperatures might have supported higher germination. In the short rains season, at Kiboko the plots sown with the certified seed and the handsorted seed sample had significantly higher germination than for all the other plots sown with all the other seed samples. The certified seed samples are usually dressed with copper based fungicides that protect the seed from infection therefore improving germination. Fungicides are used widely to improve germination in many different plant species (Neergard, 1979). Hand sorting to remove blemished and malformed seeds considerably lowered the inoculum levels resulting in improved germination. Mukunya and Keya (1975) reported higher germination percentages of bean seeds that were handsorted than those that were not

handsorted

The seed samples that had infection levels of 4.75% and 6.5% had germination percentages that were not significantly different from each other but lower than those from plots sown with other seed samples. The seedborne inoculum of *M. phaseolina* might have contributed to the lowering of the germination. Reduction in germination is probably due to the activity of the fungus at higher temperatures as reported by Kendrik (1933) and Andrus (1938). Nakawaka (1997) also reported reduced germination in cowpeas infected by *M. phaseolina*. Sclerotia develop abundantly on the seed surface of infected seeds causing rotting. Losses due to *M. phaseolina* resulted from failure of seed germination because of rapid decay by the pathogen. Dipawanita *et. al.* (1994) reported that inhibition of seed germination is by a toxin called phaseolinone and the degree of inhibition of seed germination was correlated well with the amount of toxin produced. Similarly, *Bipolaris sorokiniana* which is seedborne in barley has also been reported to lower germination (Kurppa, 1984). The seed sample with 1.0% infection and the handsorted surface sterilised seed sample had germination percentages that were not significantly different from each other. The surface sterilisation might have added value to hand sorting by lowering the inoculum level from the initial level of 6.5% to probably around 1.0% resulting in those two seedlots having similar germination percentages. Surface sterilisation with sodium hypochlorite was reported to improve germination of brassica seed infected by *Xanthomonas campestris* p. v. *campestris* (Babadoost *et. al.*, 1996). The negative and significant correlation at Kiboko between seed infection levels and germination percentages imply that low germination was recorded on plots having high levels of seed infection. Vloutoglou *et. al.* (1995) also reported a negative correlation between the proportion of seed emerged and the incidence of *Alternaria linicola* on linseed.

The correlation between seed infection level to germination was not significant at Kabete as the ambient temperatures were below the optimum for the growth of the pathogen recorded under laboratory conditions. Neergard (1979) reported that transmission of seedborne diseases is dependent on edalphic and climatic conditions. Manandhar *et al* (1998) working on rice blast caused by *P. oryzae* confirmed this as they found out that rice seedlings raised during cool weather did not develop blast

symptoms but when the conditions were favourable the seedlings developed symptoms.

At Kabete during the long rains, seed infection did not influence the post-emergence damping -off significantly but there was significant effect in the short rains season. This was probably due to the fact that the mean temperatures of 19°C were lower than the optimum temperatures for the pathogen at Kabete during the long rains season. At Kiboko the incidence of post-emergence damping-off was influenced by the level of seed infection/ contamination. Kiboko with a temperature of 30°C provided ideal temperature for the growth of the pathogen. Andrus (1938) observed similar findings that temperature appears to be a predominant factor in early seedling infection as it influences the activity of the fungus. Neergard (1979) reported that temperature has a great influence on the chemical composition and the ultramicroscopic structure of the cell walls. This might have been the case in the bean seedlings developing from seeds infected/ and or contaminated by *M. phaseolina.* Andrus (1938) working with beans reported similar findings that at warmer areas of California the disease was more severe than at cooler areas.

The plots sown with certified seed sample had post emergence damping -off that were lower than for all the other plots at Kiboko during both the long rains and the short rains but only during the short rains at Kabete. The seedborne inoculum contributed to seedling infection resulting to their death. Andrus (1938) working with beans reported that seedborne *M. phaseolina* contributed to early seedling death provided the temperatures were ideal for the growth of the pathogen. The plots sown with the certified seed sample had significantly lower seedling death than all the other plots planted with the other seed samples. The certified seed sample dressed with a copper-based fungicide could have protected the seedlings from low infection detected in agar plate test. Similarly Raut and Wangakir (1989) reported that sorghum seed dressed with fungicides had lower seedling infection caused by *M. phaseolina* than seeds that were not seed-dressed. Commercial seeds are normally dressed widely with fungicides to prevent early seedling infection on a wide variety of crops (Neegard,

1979). The plots sown with seeds that were hand sorted and handsorted surface sterilised had lower post-emergence damping- off than the plots sown with seed sample that had 6.5% infection. Hand sorting and surface sterilisation lowered the inoculum levels in the seedlots resulting in lower seedling death. There was non - significant correlation between seed infection levels and the incidence of post-emergence damping off. The level of post-emergence could not be predicted from the level of seed infection as some seeds failed to germinate as they decayed as result of the activity of the fungus (Dipawanita *et al.*, 1994).

The incidence of charcoal rot at V 3 and R8 in plots sown with seed having different levels of infection were higher for both seasons at Kiboko and during short rains at Kabete. The plots sown with the seed samples with 6.5% and 4.75% infection level had higher incidence of charcoal rot due to the presence of seed borne inoculum. The plots sown with the certified, handsorted, handsorted and surface sterilised seed samples had lower incidence of charcoal rot at V3and R8 as the seeds were relatively free of seedborne inoculum. This is in agreement with Isanda (1995) who reported higher incidence of bean anthracnose caused by *Colletotrichum lindemuthianum* in plots that were sown with bean seeds containing high levels of seedborne inoculum and lower levels of disease in plots that were sown with certified seeds. Similar findings were reported by Mukunya and Keya (1978) who reported low incidences of diseases in plots that were sown with handsorted bean seed samples. Songa (1995) reported that surface sterilisation reduced the level of seedborne *M. phaseolina* and recommeded that this can be used as a measure in the control of charcoal rot. During the long rains, the ambient conditions were not ideal for the growth and proliferation of *M. phaseolina* and therefore the incidence of charcoal rot at V3 and R8 in plots sown with seed having different levels of infection levels were not different. Manadhar (1998) also reported that rice grown in areas where cool climate prevails did not develop blast symptoms despite having high incidences of seedborne *Pyricularia oryzae*.

The positive and significant correlation between the seed infection level and incidence of charcoal rot at V3 and R8 at Kiboko for both seasons and at Kabete

during the short rains imply that high incidence of seed borne *M. phaseolina* translated to high level of disease in the field. A similiar relationship was reported by Isanda (1995) who reported higher incidences of bean anthracnose caused by *Colletotrichum lindemuthianum* in plots that were sown with bean seeds containing high levels of seedborne inoculum. The non-significant correlation between the seed infection level and incidence of charcoal rot at V3 and R8 at Kabete during the long rains can be explained by the fact that ambient conditions were not ideal for the growth and proliferation of *M. phaseolina*. These findings confirm earlier reports by Sattar (1933) and Neergard (1979) who reported that the expression of diseases are influenced greatly by the prevailing climatic conditions.

Results indicate that the plots sown with seedlots with seeds having infection levels of 6.5% and 4.75% had higher severity when compared to all the other plots. The plots sown with the seedlots that had low infection (1.0%), the hand sorted and the handsorted surface sterilised seed samples had disease severity that was not different from each other. Isanda (1995) similarly reported that high amount of seed borne inoculum resulted in high levels of bean anthracnose caused by *C. lindemuthianum*. Handsorting and surface sterilisation considerably lowered the initial inoculum therefore resulting in lower disease incidence and severity in those plots. Hand sorting to remove malformed and discoloured seeds considerably freed the seedlot from higher infection level of inoculum resulting in reduced disease levels. Mcdonald (1959) reported similar findings in rape seed infected by *Alternaria brassicae* where lower disease ratings were recorded in plots that were sown with properly cleaned seeds. Neergard (1979) reported that in Britain, a machine that is able to separate discoloured diseased seeds from normal health seeds electronically has been designed. This procedure has been successfully used for the pea seed visibly infected by *Aschochyta* spp. and *Colletotrichum lindemuthianum* on white *Phaseolus* bean.

Surface sterilisation of the hand sorted sample did not have a significant effect on the level of disease in the field. Songa (1995) reported that surface sterilisation also

reduced the inoculum by one half and therefore recommended it as a control method towards the management of charcoal rot. Kabeere *et. al.* (1997) also reported lower incidences of *Fusarium graminearum* and *Fusarium subglutinans* from maize seeds that were surface sterilised than those that were non- surface sterilised. However surface sterilisation in this work appears to have no added advantage over handsorting of seed material. The inoculum might have been deep seated in the cotyledons and therefore was not eliminated by surface sterilising.

The severity of charcoal rot cumulatively increased with the time of sampling reaching a maximum peak 8 weeks after sowing. Majority of seedborne plant diseases are reported to increase with time and reach a peak provided the prevailing weather conditions are ideal for growth of the pathogens (Neergard, 1979). Origa (1992) confirmed such a trend in the spread of halo blight caused by *Pseudomonas phaseolicola*. Such a trend was further confirmed by Isanda (1995) who was working on bean anthracnose caused by *Colletotrichum lindemuthianum.* The plots at Kiboko had generally higher levels of severity than those at Kabete during both the long and short rains. The temperatures prevailing at Kiboko are seen to be ideal for the growth and seed transmission of charoal rot. At Kabete the severity levels recorded for all the plots sown with different seed samples were not significantly different during the short rains and long rains. However there was slight increase of charcoal rot severity during the short rains. The temperatures prevailing during the season were not very ideal for the growth of the pathogen as transmission of seedborne pathogen *M. phaseolina* is determined by the prevailing weather and climatic conditions (Neergard, 1979). The significant interaction between time of sampling with site and season imply that the environment and the season had a significant effect on the progress of charcoal rot resulting in higher disease ratings at Kiboko during the short rains with low ratings of charcoal rot at Kabete during the long rains.

The yield/ha and percentage yield reduction /ha obtained from plots that were planted with seedlots that had different levels of infection /and or contamination were not different both at Kabete and at Kiboko. At Kabete, the levels of disease

transmission from infected seeds was too low as to have any effect on the yield. At Kiboko there were no differences in yields among plots planted with seed samples having different levels of seedborne *M. phaseolina* despite the high levels of disease tranmission. Neergard (1979) reported no yield differences from different levels of seed infection by *Drechslera caternaria* and *Drechslera dictyoides* in rye grass, *Lolium muiltflorium* and *Lolium perenne*. *M. phaseolina* causes loss in germination and early seedling loses leaving spaces between the plants. Due to increased spacing the plants are able to compensate this loss during the growth period of the plants resulting in negligible loss differences in yield. Stand reducers are likely to widen the variation in plant performance in the population. Plants are likely to compensate fully for the effect of seedling establishment even over wide ranges. Compensation by adjacent plants may be more important to crop yield in these plants than with average or reduced performance (Gaunt, 1995). The negative and significant correlation between seed infection and germination can therefore be explained in that seedlots that had high infection levels of *M. phaseolina* had lower germination resulting in low plant density. The correlation between seed infection and yield was not significant indicating that there was no influence of seed infection on the yield obtained from plots. Losses due to early seedling infection might have been compensated for during the growth period of the bean crop resulting in non-significant losses in yield (Gaunt, 1995).

The yields recorded at Kabete were about two times that recorded at Kiboko. Yield potential is correlated with duration of crop growth (Tanaka, 1983). The beans planted at Kiboko with higher prevailing temperature than at Kabete had a shorter growth period and this translated to lower yields as the plants had less time to accumulate photosynthate products. The same explanation could be applied to the higher yields recorded during the long rains where the growth period of the crop was longer due to slightly lower temperatures than during the short rains.

5:1:5 Plant inoculation and subsequent seed contamination and / or infection

Neergard (1979) reported that the degree of infection of seed may be related to the time of infection as defined by the growth stage of the host. He asserted that the exact

time of infection within this period determined the extent of infection of individual seeds and number of seeds infected. Bean plants inoculated at planting with colonised rice grains resulted in higher incidences of subsequent seed contamination/ infection by *M. phaseolina*. Inoculations carried out at planting, at two and at four weeks after sowing resulted in significantly higher infection/and or contamination than when inoculations were carried out later in the growth stages. When inoculations are done at early stages of growth, the disease has more time to develop and establish in the plant and infect the developing seeds. Plants infected four weeks after sowing may not produce infected seed. This is in agreement with findings reported by Origa (1992) who inoculated beans with *Pseudomonas syringe* pv *phaseolicola* and Isanda (1995) who inoculated beans with *Colletotrichum lindemuthianum*.

Neergard (1979) noted that plants as well as their infectious diseases are generally distributed geographically according to climatic conditions and also climatic conditions leading to severe field attacks by seedborne pathogens usually result in increased seedborne inoculum. This is in agreement with the findings that seeds harvested from Kabete had lower chances of infection/ and or contamination levels than seeds from Kiboko as conditions prevailing in Kiboko were more ideal for the development of the pathogen. The average temperatures of 28°C prevailing at Kiboko during the long rains was probably more ideal for the proliferation of the pathogen within the host tissues resulting in higher transmission to the seed than during the short rains when the average temperatures were 32°C. This is in agreement with Frosheiser (1974) who reported that the transmission of alfalfa mosaic virus was much less at 29°C than at 24°C.

Plots inoculated with colonised rice grains at planting had significantly lower yields than all the other plots at Kiboko during the short rains. This is in agreement with Isanda (1995) who reported that early inoculations of beans with *Colletotrichum lindemuthianum* resulted in lowest yields. Green and Lee (1989) working with peanuts found out that early inoculations of peanuts with Peanut Stripe Virus (pstv) resulted in the lowest yields. This can be explained by effect of the disease on amount of photosynthesis. The yields from plots inoculated at pod filling stage, at maturity and inoculated with sterile toothpicks were not significantly different from each other.

Bolton (1974) and Ayers *et. al.* (1979) reported that maize inoculated with *Drechslera maydis* race T, after the dough stage, had disease levels with little or no effect on the rate of accumulation or the amount of photosynthate translocated to the grain. The same could have occurred where bean plants were inoculated with *M. phaseolina.* At Kiboko during the long rains and at Kabete for both seasons there were no significant differences in yields in plots inoculated at different stages of growth. Losses due to early seedling infection might have been compensated for during the growth period of the bean crop resulting in non-significant losses in yield (Gaunt, 1995). The positive and significant correlation between time of inoculation and subsequent seed infection at Kiboko imply that the earlier the plants are inoculated the higher the level of subsequent seed infection. The earlier the plants are inoculated the more time the pathogen has to grow and establish within the tissues of the host (Origa, 1992; Isanda, 1995).

CONCLUSIONS AND RECOMMENDATIONS

The present work provides useful information on the growth conditions of the pathogen, the ideal seed health technique for *M. phaseolina* and the prevalence of seedborne *M. phaseolina* on seeds from different areas of Kenya. Investigations were conducted on the influence of seedborne inoculum on the incidence and severity of *M. phaseolina*. Studies were also carried out on the influence of growth stage at which inoculation infection could become crucial in the subsequent seed infection/ contamination of the harvested seed.

Macrophomina phaseolina can grow well on several growth media particularly on PDA and MEA. Although growth of the pathogen occurred on BSDA, this was fairly limited compared to the former two media. Under laboratory conditions the growth of the pathogen was found to be temperature dependent with an optimum range of 30-35°C. In contrast photoperiodism had no significant effect on the growth of *M. phaseolina* in this study and thus in the development of seed health testing procedures, it may be advisable to use MEA or PDA at temperatures between 30-35°C.

Among the various methods used for seed health testing, direct plating of seeds on PDA gave the highest incidences of *M. phaseolina*, direct plating on blotter resulted in fairly high incidences while the dry seed examination gave the least level of infection. While direct plating of seed on PDA was very sensitive it is however unsuitable as it is expensive in terms of media, petri-dishes and time taken in media preparation. There is also quick growth of saprophytic and other pathogenic fungi that hamper rapid identification of the pathogen. The dry seed examination method can be used to get the general idea about the health status of a particular seed lot. However it is recommended that this method is combined with direct plating of seed blotter as the latter is cheap in terms of material used and identification of the pathogen is easy and straight forward. The method can conviniently be carried out in laboratories where even basic facilities are lacking.

The pathogen *M. phaseolina* was found in seed samples collected from the three study districts namely Kakamega, Kiambu and Machakos. However, the

incidence of seed borne *M. phaseolina* was distinctly high in samples obtained from Machakos. It appears therefore that the pathogen is more common in hot and dry environments. The seed sample obtained from Kakamega Municipality was free of seed infection/contamination by *M. phaseolina*. This seed sample can therefore be multiplied as a source of seeds free from infection/ contamination by *M. phaseolina*. The level of seedborne *M. phaseolina* was higher in farmers seeds compared with levels found in certified seeds. This suggests that using certified seed is advisable as a measure to reduce the disease epidemic under bean production systems. However since most of our farmers are resource poor and such seed is often out of reach, they should be encouraged to hand sort their seeds to remove blemished and malformed seeds as the measure clearly reduced the disease in the field. This method has been successfully applied in the control of bean anthracnose in white *Phaseolus* bean.

The high incidences and severity of charcoal rot at Kiboko and during the short rains imply that the disease is more prevalent in hot dry areas and it is usually more severe during the short rains season which apparently is the season farmers that in Machakos rely on. Therefore existing recommended control measures for charcoal rot using cultural or host resistance methods are applied to reduce crop losses.

Investigations on timed inoculations and the resultant seed infection showed that the plants exhibiting charcoal rot symptoms early in life often end up with highly infected/contaminated seeds. In contrast, plants that are infected with charcoal rot later in life result in low incidence of *M. phaseolina*. This implies that in seed production rouging of the former may contribute significantly to high quality seed.

The high levels of seed infection /contamination recorded in seeds harvested at Kiboko during the long rains imply that for the control of *M. phaseolina*, the seed crop should be grown during the short rains. It is therefore recommended -:

1. Further work on this pathogen should focus on setting of tolerance levels for *M. phaseolina* certified seeds.
2. Other control measures to control seed borne *M. phaseolina* should be investigated.

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APPENDICES

Appendix 1(i) Development stages of the common bean plant.

Stage Description

V0. Germination: Water absorption by the seed, emergence of the radicle, and transformation into the primary root.

V1 Emergence: Cotyledons appear at soil level and begin to separate. The epicotyl initiates development.

V2 Primary leaves: Totally opened primary leaves.

V3. First trifoliate leaf: The first trifoliate leaf opens and second trifoliate leaf appears.

R5 Pre: The first flower bud or the first raceme appears. Flower buds in determinate varieties are formed on the last stem or branch node. In indeterminate varieties racemes are first observed on lower nodes.

R6 Flowering: The first flower opens.

R7 Pod formation: The first pod appears being more than 2.5cm long.

R8. Pod filling: The first pod begins to fill (seed growth) At the end of the stage the seeds lose their green colour and begin to show varietal characteristics. Defoliation initiates.

R9: Physiological maturity: Pods lose their pigmentation and begin to dry. Seeds develop their typical varietal colour.

a. V = vegetative; R=reproductive.

b. b. When evaluating populations, each stage begins when 50% of the plants show the conditions correspond to the description.

(Source: CIAT publication, 1987)

Appendix (ii) Control of Storage Pests

Actellic (a.i piriphos- Methyl, W/W = 1%) is an organophosphorous insecticide commonly used for the control of storage insect pests of cereals. Therecommended rate of application in bean seed is about 2.2 g/10,000 bean seeds but half of this rate was succesfully used to control bruchids (*Acathosclidis obectas*). There was

therefore some important need to know if this chemical could have any effect on *M. phaseolina* both on the surface and inside the seeds. The *in- vitro* test was carried out and the results shown below.

	Wt of Actellic\100 ml of Potato Dextrose Agar		
	0.0	0.2	1.2
Radial growth of *M. phaseolina*	+	+	+

The results show that Actellic did not have any effect on *M. phaseolina*. Therefore of its application to control dry bean bruchids did not have a negative effect on the results expected from the analysis.

Appendix 2. Anova for effect of growth media. temperature and light regime on mycelial radial growth (mm) of *M. phaseolina*.

Source	SS	DF	MSS	F	P
Main effects					
Media(M)	17504.58	2	8752.29	1456.92	***
Light(L)	7.51	2	3.76	0.63	ns
Temp.(T)	13099.01	4	3274.75	545.12	***
Interaction					
MxL	200.98	4	50.24	8.36	***
MxT	4369.13	8	546.14	90.91	***
LxT	147.97	8	18.50	3.08	***
MxTXL	363.10	16	22.69	3.78	***
Error	540.67	90	6.01		
Total	302332.93	134			

Appendix 3

ANOVA for seed health analysis

Source	DF	SS	MSS	F	P
District	2	4840.50	2420.25	253.38	***
Site	12	762.60	69.33	7.26	***
Method	5	3329.38	665.88	69.7	***
Error	845	8071.21	9.55		
Total	863	17003.69			

C.V.(%) =127.21 S.E.=3.09

Appendix 4

Anova showing the effect of seed infection in germination at Kiboko

(a) long rains season

Source	Df	SS	MSS	F	P
Blocks	3	0.338	0.112	1.760	ns
Seed infection	5	9.764	1.953	30.54	***
Error	15	0.959	0.063		
Total	23	11.061			

C.V.= (%) 12.58 S.E=0.252

(b) short rains season

Source	Df	SS	MSS	F	P
Blocks	3	1.04	0.35	1.66	ns
Seed infection	5	2.60	0.52	2.49	***
Error	15	3.14	0.21		
Total	23	6.78			
C. V. (%)=27.86	S.E.=0.46				

Appendix 5

Anova showing the effect of seed infection in germination at Kabete

(a) long rains season

Source	Df	SS	MSS	F	P
Blocks	3	1.042	0.347	1.66	ns
Seed infection	5	2.602	0.520	2.49	ns
Error	15	3.14			
Total	23	6.78			
C.V.(%) =27.86	S.E.=0.46				

(b) short rains

Source	Df	SS	MSS	F	P
Blocks	3	1.042	0.347	1.66	ns
Seed infection	5	2.602	0.520	2.49	ns
Error	15	3.14			
Total	23	6.78			
C.V.(%) =27.86	S.E.=0.46				

5(c) Anova for the effect of levels of seed infection, season and site on germination of various seedlots

Source	SS	df	MS	F	P
Blocks	96.34	3	32.12	3.75	*
TREA (T)	1416.53	5	283.31	33.08	***
SITE (S)	376.04	1	376.04	43.90	***
SEAS (SE)	17.51	1	17.51	2.04	ns
Interaction					
TREA x SITE	72.52	5	14.50	1.69	ns
TREA x SEAS	76.93	5	15.39	1.80	ns
SITE x SEAS	287.04	1	287.04	33.51	***
T x S x SE	30.46	5	6.10	0.71	ns
Error	591.03	69	8.58		
Total	2964.41	95			
C.V.(%)=3.43	S.E.=1.46				

Appendix 6

Anova showing the effect of seed infection to post emergence damping off at Kiboko

(a) long rains

Source	Df	SS	MSS	F	P
Blocks	3	0.69	0.23	1.59	ns
Seed infection	5	11.07	2.21	15.49	***
Error	15	2.15	0.14		
Total	23	13.91			
C. V.=(%) 12.71	S.E.=0.38				

(b) short rains season

Source	Df	SS	MSS	F	P
Blocks	3	0.68	0.26	1.15	ns
Seed infection	5	6.27	1.25	6.39	***
Error	15	2.90	0.20		
Total	23	9.95			

C.V.=(%) 11.99 S.E.=0.44

Appendix 7

Anova showing the effect of seed infection to post emergence damping off at Kabete

(a) long rains season

Source	Df	SS	MSS	F	P
Blocks	3	0.23	0.08	0.53	ns
Seed infection	5	0.24	0.05	0.83	ns
Error	15	1.37			
Total	23	1.84			

C.V.=23.67% S.E.=0.30

(b) short rains

Source	Df	SS	MSS	F	
Blocks	3	0.39	0.13	2.12	ns
Seed infection	5	25.48	5.10	84.20	***
Error	15	0.91	0.061		
Total	23	26.78			

C.V.= (%) 7.52 S.E=0.25

(c) Anova for the effect of level of seed infection, season and site on post-emergence damping-off

Source	SS	df	MS	F	P
Blocks	2.90	3	0.97	0.48	ns
TREAT (T)	249.84	5	49.98	24.71	***
SITE (S)	0.13	1	0.13	0.06	ns
SEAS (SE)	172.00	1	172.00	85.06	***
Interaction					
T x S	6.54	5	1.31	0.65	ns
T x SE	126.87	5	25.37	12.55	***
S x SE	2.50	1	2.50	1.24	ns
T x S x SE	8.61	5	1.72	0.85	ns
Error	139.53	69	2.02		
Total	708.91	95			

C.V.(%)=44.4 S.E.=0.71

Appendix 8

Anova showing the effect of seed infection on incidence of charcoal rot at V3 at Kiboko

(a) long rains season

Source	Df	SS	MSS	F	P
Blocks	3	3.43	1.14	7.68	***
Seed infection	5	8.76	1.75	11.77	***
Error	15	2.23	0.15		
Total	23	14.42			

C. V.= (%) 9.57 S.E= 0.39

(b) short rains season

Source	Df	SS	MSS	F	P
Blocks	3	0.56	0.19	2.33	ns
Seed infection	5	5.06	0.08	12.57	***
Error	15	1.21	0.08		
Total	23				

C.V.=7.75% S.E. =0 29

Appendix 9

Anova showing the effect of seed infection on incidence at V3 at Kabete during the

(a) long rains season

Source	Df	SS	MSS	F	P
Blocks	3	0.39	0.13	2.12	ns
Seed infection	5	0.77	0.154	0.225	ns
Error	15	0.52	0.03		
Total	23				

C.V.(%)=6.67 S.E.=0.08

(b) short rains season

Source	Df	SS	MSS	F	P
Blocks	3	0.06	0.02	0.33	ns
Seed infection	5	2.62	0.52	8.48	***
Error	15	0.93			
Total	23	3.61			

C.V.=15.55% S.E=0.25

(c) ANOVA for the effect of level of seed infection. season and site on charcoal-rot incidence at V3

Source	SS	df	MS	F	P
Blocks	36.15	3	12.05	2.76	*
TREAT (T)	1622.58	5	324.52	74.24	***
SITE (S)	16.67	1	16.67	3.81	ns
SEAS (SE)	1650.04	1	1650.04	377.49	***
Interaction					
T x S	55.08	5	11.02	2.52	*
T x SE	818.33	5	163.67	37.44	***
S x SE	3.38	1	3.38	0.77	ns
T x S x SE	25.00	5	5.00	1.14	ns
Error	301.60	69	4.37		
Total	4528.83	95			

C.V.(%)=31.2 S.E.=1.05

Appendix 10. Anova showing the effect of seed infection on percent charcoal rot incidence at R8 at Kiboko

(a) Long rains

Source	DF	SS	MSS	F	P
Block	3	244.13	81.34	3.72	***
Seed infection	5	1669.71	233.94	5.25	***
Error	15	328.13	21.88		
Total	23	2241.96			

C.V.(%)=68.6 S.E.=9.06

(b) short rains

Source	SS	df	MS	F	P
Blocks	2.83	3	0.94	0.04	ns
Seed infection	9025.50	5	1805.10	78.67	***
Error	344.16	15	22.94		
Total	9372.59	23			

C.V.=15.6 S.E.=2.4

Appendix 11. Anova showing the effect of seed infection on charcoal rot incidence at R8 at Kabete

(a) Long rains

source	SS	df	MS	F	P
Blocks	149.36	3	49.79	2.17	ns
Seed infection	49.09	5	9.82	0.43	ns
Error	343.70	15	22.91		
Total	542.16	23			

C.V.(%)=30.8 S.E=2.4

Appendix12 Anova to show the progress of charcoal rot at Kiboko

(a) long rains

source	SS	df	MSS	F	P
Blocks	1.08	3	0.36	0.10	ns
Seed infection	119.75	5	23.95	6.73	***
Prd of sampling	96.33	3	32.11	9.02	***
PrdXseed infection	191.67	15	6.11	3.58	***
Error	128.18	36			
Total	537.01				

C.V.(%)=12.22

(b) short rains

source	SS	df	MSS	F	P
Blocks	8.83	3	1.37	0.10	ns
Seed infection	145.75	5	13.4	40.6	***
Prd of sampling	102.08	3	15.74	46.7	***
PrdXseed infection	177.67	15	5.50	3.58	***
Error	11.76	36	0.329		
Total	537.01				

C.V.(%)=18.16

Appendix 13 Anova to show the progress of charcoal rot at Kabete

(a) long rains

source	SS	df	MSS	F	P
Blocks	2.56	3	0.85	1.04	ns
Seed infection	9.25	5	1.85	2.27	ns
Prd of sampling	19.64	3	6.55	8.03	***
PrdXseed infection	8.52	15	0.68	0.695	ns
Error	291.38	36	0.84		
Total	331.35				

C.V. (%)=16.9

(b) short rains

source	SS	df	MS	F	P
Blocks	3.02	3	1.00	1.05	ns
Seed infection	6.78	5	1.36	1.43	ns
Prd of sampling	21.55	3	7.18	7.53	ns
PrdXseed infection	7.92	15	0.53	0.55	ns
Error	34.22	36	0.95		
Total	73.49				

C.V. (%)=3.31

(c) Source	df	SS	MSS	F	P
Blocks	3	7.35	2.45	40.5	*
Seed infection levels	3	6.29	2.10	34.7	*
Period after sowing	3	15.33	5.11	84.5	*
Season	1	12.11	12.11	200.07	*
Site	1	16.11	16.11	266.37	*
Seed infectionxprd after sowing	9	9.08	1.01	16.7	*
Seed infectionxsite	3	8.90	3.00	49.6	*
Seed infectionxseas	3	11.2	3.73	54.56	*
Prd after sowingxseas	3	14.2	4.73	71.1	*
Prd after sowingxsite	3	17.59	5.86	26.3	*
Prd after sowingxseasxsite	3	25.32	2.81	46.4	*
seaxsitexseed infection	3	15.33	5.11	84.5	*
seaxsitexseed infectionx prd after sowing	9	25.32	0.60		
Error	125	7.56			
Total	166	151.04			

C.V.(%)=2.58

Appendix 14

Severity score (percent of stem damage) of charcoal rot caused by *M. phaseolina* taken every 2 weeks in plots sown with seed samples having different levels of infection/contamination (N=20).

(a) long rains season at Kiboko

Time of sampling Seed infection level	14	28	42	56
1	20.7	28.5	32.5	34.5
2	31.0	32.2	34.5	35.8
3	33.5	39.8	42.8	45.7
4	19.3	28.7	31.8	32.4
5	17.5	25.4	26.2	29.2
6	16.5	18.8	20.1	22.2
mean	23.1	29.0	31.3	33.3

(b) Short rains season at Kiboko

Time of sampling Seed infection level	2	4	6	8
1	21.8	27.8	32.2	44.9
2	33.3	30.1	38.2	41.1
3	33.3	39.5	44.8	44.1
4	16.2	25.7	30.5	38.5
5	17.3	26.8	28.3	32.3
6	9.5	17.9	23.3	29.7
mean	21.9	28.1	32.1	38.5

c) long rains at Kabete

Time of sampling Seed infection level	2	4	6	8
1	8.5	9.20	11.2	12.3
2	9.2	9.80	14.1	16.1
3	13.2	14.5	15.6	17.2
4	10.1	11.0	12.2	13.2
5	10.5	11.2	12.1	15.2
6	9.5	11.1	13.2	13.6
mean	10.2	11.3	13.1	14.7

(d) Short rains season at Kabete

Time of sampling Seed infection level	14	28	42	56
1	11.3	13.5	16.3	19.3
2	13.3	16.2	21.2	24.3
3	11.2	22.3	24.7	27.6
4	13.9	14.6	16.2	19.6
5	13.3	13.9	17.0	17.0
6	12.8	12.2	11.5	17.5
mean	12.6	15.5	17.8	20.9

Appendix 15. ANOVA for yield recorded at Kiboko plots sown with seed having different infection level

(a) long rains

Source	DF	SS	MSS	F	P
Block	3	504797.92	168265.97	2.62	ns
seed infection level	5	182702.86	36540.57	0.57	ns
Error	15	962088.80	64139.25		
Total	23	1.649.589.58			
S.E.=37.91		C.V.=32.79%			

(b) short rains

Source	SS	df	MSS	F	P
Blocks	504797.92	3	168265.97	2.62	ns
Seed infection level	182702.86	5	36540.57	0.57	ns
Error	962088.80	15	64139.25		
Total	1649589.58	23			
S.E.=74.56	C.V.=39.98%				

15(c) Anova for the effect of season and site on charcoal-rot incidence at R8

Source	SS	df	MS	F	P
Blocks	54.55	3	18.18	1.08	ns
TREAT	3676.96	5	735.39	43.57	***
SITE (S)	3.96	1	3.96	0.23	ns
SEAS (SE)	15567.77	1	15567.77	922.42	***
Interaction					
T x S	2336.21	5	467.24	27.69	***
T x SE	1390.27	5	278.05	16.48	***
S x SE	0.32	1	0.32	0.02	ns
T x S x SE	1671.98	5	334.40	19.81	***
Error	1164.51	69	6.88		
Total	25866.54	95			
C.V.(%)=13.76	S.E.=1.31				

Appendix 16. ANOVA for yield recorded at Kabete plots sown with seed having different infection level

(a) long rains

Source	DF	SS	MSS	F	P
Block	3	612341.34	204113.78	0.01	ns
Seed infection level	5	2126105.80	425221.16	0.00	ns
Error	15	633680168.40	42245344.56		
Total	23	636418615.60			
S.E=32.49	C.V.(%)=22.56				

(b) short rains

Source	SS	df	MS	F	P
Blocks	38515.97	3	12838.66	0.39	ns
Seed infection level	157034.91	5	31406.98	0.95	ns
Error	497878.91	15	33191.93		
Total	693429.78	23			
C.V.(%) =45.77	S.E=32.44				

(c) ANOVA for the effect of level of seed infection. season and site on yield.

Source	SS	df	SS	F	P
Blocks	5.38	3	1.79	0.12	ns
TREAT (T)	1739.92	5	347.98	23.78	***
SITE (S)	1078.70	1	1078.70	73.72	***
SEAS (SE)	2024.00	1	2024.00	138.32	***
Interaction					
T x S	227.20	5	45.44	3.11	** *
T x SE	230.52	5	46.10	3.15	***
S x SE	874.83	1	874.83	59.78	***
T x S x SE	295.99	5	59.20	4.05	**
Error	1009.70	69	14.63		
Total	7486.26	95			
C.V.(%)=0.35	S.E.=1.91				

Appendix 17. ANOVA for yield reduction recorded at Kiboko plots sown with seed having different infection level

(a) long rains

Source	SS	df	MS	F	P
Blocks	149.36	3	49.79	2.17	ns
Seed infection	49.09	5	9.82	0.43	ns
Error	343.70	15	22.91		
Total	542.16	23			

C.V.(%)=35.4 S.E.=2.39

(b) short rains

Source	DF	SS	MSS	F	P
Block	3	0.79	0.26	0.53	ns
Seed infection	5	5.71	1.14	2.29	ns
Error	15	7.46	0.50		
Total	23	13.96			

C.V.(%)=3.03 S.E.=0.35

Appendix 18. ANOVA for yield reduction recorded at Kabete plots sown with seed having different infection level

(a) short rains

Source	DF	SS	MSS	F	P
Block	3	97.46	32.49	2.90	***
Seed infection	5	72.71	14.54	1.30	ns
Error	15	167.79	11.18		
Total	23	337.96			

C.V.(%)=40.6 S.E.=1.67

(b) long rains

Source	SS	df	MSS	F	P
Blocks	149.36	3	49.79	2.17	ns
Seed infection	49.09	5	9.82	0.43	ns
Error	343.70	15	22.91		
Total	542.16	23			

C.V.(%)=33.47 S.E.=2.39

(c)

Source	SS	df	SS	F	P
Blocks	5.38	3	1.79	0.12	ns
TREAT (T)	1739.92	5	347.98	23.78	***
SITE (S)	1078.70	1	1078.70	73.72	***
SEAS (SE)	2024.00	1	2024.00	138.32	***
Interaction					
T x S	227.20	5	45.44	3.11	*
T x SE	230.52	5	46.10	3.15	*
S x SE	874.83	1	874.83	59.78	***
T x S x SE	295.99	5	59.20	4.05	**
Error	1009.70	69	14.63		
Total	7486.26	95			

C.V.(%)=13.2 S.E.=1.91

Appendix 19. ANOVA for yield after inoculation at different growth stages at Kiboko

(a) long rains

Source	SS	df	MS	F	P
Blocks	33145	2	16577.00	2.71	ns
Growth stage	42347	6	7067.80	1.15	ns
Error	73421	12	61184.42		
Total	148922	20			
S.E=35.34		C.V.= 46.17 %			

(b) short rains

Source	SS	df	MS	F	P
Blocks	10152.70	2	5076.35	0.50	ns
Growth stage	43250.10	6	8650.02	8.85	***
Error	102303.28	12	0230.33		
Total	155706.07	20			
S.E=18.2		C.V.=26.6%			

Appendix 20. ANOVA for yield after inoculation at different growth stages at Kabete.

(a) long rains

Source	SS	df	MSS	F	P
Blocks	137836.76	2	68918.38	1.80	ns
Inn	276945.83	6	46157.64	1.21	ns
Error	459455.95	12	38288.00		
Total	874238.54	20			
S.E=97.8		C.V.(%)=9.6			

(b) short rains

Source	SS	df	MS	F	P
Blocks	335306.40	2	167653.20	9.57	**
Growth stage	18852.98	6	3142.16	0.18	ns
Error	210158.19	12	17513.18		
Total	564317.56	20			
S.E=66.17		C.V.(%)=47.7			

(c)

ANOVA for the effect of level of seed infection. season and site on yield

Source	SS	df	SS	F	P
Blocks	5.38	3	1.79	0.12	ns
TREAT (T)	1739.92	5	347.98	23.78	***
SITE (S)	1078.70	1	1078.70	73.72	***
SEAS (SE)	2024.00	1	2024.00	138.32	***
Interaction					
T x S	227.20	5	45.44	3.11	** *
T x SE	230.52	5	46.10	3.15	** *
S x SE	874.83	1	874.83	59.78	***
T x S x SE	295.99	5	59.20	4.05	**
Error	1009.70	69	14.63		
Total	7486.26	95			
C.V.(%)=2.98	S.E.=1.91				

Appendix 21. ANOVA for resultant seed infection\contamination on harvested seeds at Kiboko

(a) Long rains

Source		DF	SS	MSS	F	P
Block		2	0.40	0.20	14.39	***
Growth stage		6	9.35	1.56	5.84	***
Error	10		1.30	0.11		
Total	17		11.06			
S.E=0.19		C.V.=26.01%				

(b) Short rains

Source	DF	SS	MSS	F	P
Blocks	2	7.46	2.49	0.17	ns
Growth stage	6	196.21	39.24	4.72	***
Error	12	216.29	14.42		
Total	20	419.96			
S.E=3.10		C.V.=39.8%			

Appendix 22. ANOVA for resultant seed infection\contamination on harvested seeds at Kabete

(a) long rains

Source	DF	SS	MSS	F	P
Block	2	0.07	0.04	0.10	ns
Growth stage	6	0.73	0.12	0.35	ns
Error	12	4.12	0.34		
Total	20	5.57			
S.E=0.48	C.V.=1.82%				

(b) short rains

Source	DF	SS	MSS	F	P
Blocks	2	0.79	0.26	0.53	ns
Growth stage	6	6.71	1.34	2.70	ns
Error	12	7.46	0.50		
Total	20	14.96			
S.E=0.58	C.V.=1.12				

(c) ANOVA for the effect of stage of growth infection on level of infection

Source	SS	df	SS	F	P
Blocks	5.38	3	1.79	0.12	ns
TREAT (T)	1739.92	5	347.98	23.78	***
SITE (S)	1078.70	1	1078.70	73.72	***
SEAS (SE)	2024.00	1	2024.00	138.32	***
Interaction					
T x S	227.20	5	45.44	3.11	*
T x SE	230.52	5	46.10	3.15	*
S x SE	874.83	1	874.83	59.78	***
T x S x SE	295.99	5	59.20	4.05	**
Error	1009.70	69	14.63		
Total	7486.26	95			

C.V.(%)=13.2 S.E.=1.91

App. 23

Environmental conditions during the cropping seasons

(a)(i)Kabete

	April	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec	Jan.
Rainfall(mm)	32.2	29.7	17.2	11.9	11.8	12.3	44.4	34.5	0	0
Temperature (^{0}C)	16.0	18.3	16.6	15.6	15.4	17.4	19.2	23.2	22.6	23.1

(b) Kiboko

	April	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec	Jan.
Rainfall(mm)	10.2	12.7	2.2	1.9	1.8	2.1	17.4	14.0	0	0
Temperature (^{0}C)	26.0	28.3	28.6	26.6	31.4	32.4	30.2	33.2	32.6	33.1