CHAPTER 1

INTRODUCTION

Seed is probably the most important single input for arable cultivation that determines the potential production and thus productivity of all other inputs (Friis-Hansen, 1995). About 90 % of the world food crops including sorghum (*Sorghum bicolor* (L) Moench) are propagated by seed (Maude, 1996). However, seeds can be passive carriers of pathogens that are transmitted when the seed hosts are sown and emerge under suitable environmental conditions. Fungi, bacteria, viruses and nematodes can be carried with, on or in the seeds resulting in tremendous yield losses (Neergaard, 1979).

Sorghum is affected by a range of fungal seed borne diseases including ergot (*Claviceps africana*), seed rot (*Fusarium moniliforme*), zonate leaf spot (*Gloeocercospora sorghi*), downy mildew (*Sclerospora sorghi*), loose smut (*Sphacelotheca cruenta*), covered smut (*Sphacelotheca sorghi*), leaf spots (*Phoma sorghina), Bipolaris bicolor*, anthracnose (*Colletotrichum graminicola*) and grey leaf spot (*Cercospora* sp.) (Almekinders and Louwaars, 1999; Kaula and Chisi, 2002 and Neergaard, 1979). Of all these diseases, smuts are the most destructive. Therefore, seed health testing is a prerequisite to minimise loses by assessing the quality of seed before it is sown [International Seed Testing Association (ISTA), 1985].

Most smallholder farmers that venture into sorghum production use farm saved seed, which is likely to be of poor quality. This is because certified seed is expensive and most farmers can hardly afford purchasing it. Sometimes, the economic gains from using a higher seed quality do not justify the purchase of seed (Almekinders and Louwaars, 1999). In most cases, it is likely that seed borne pathogens accumulate in farm saved seeds with time.

Seed health testing constitutes part of the seed certification and plant quarantine practices aimed at reducing the distribution of seed borne pathogens by both national and international trade of seeds (Mathur and Manandhar, 1993). However, seed health is of great concern especially to farmers and the seed producing agencies in tropical and subtropical countries (Mathur and Kongsdal, 2000). Mathur, 1983 noted that seed certification schemes do not exist in some tropical countries. In Zimbabwe, seed certification is practiced by the Seed Services Institutes under the Department of Agricultural Research and Extension (AREX) but testing for seed quality mainly covers purity analysis, germination tests, moisture determination and trueness to variety. Seeds are seldom tested routinely for seed borne diseases but on request usually as a result of low germination percentage (O. Randi (2004) [1], personal communication). Additionally, little attention has been paid to the transmission of pathogens from seed to plant and eventually to seed. This is done using the seedling symptom test that helps demonstrate the seed borne nature of fungi that are able to attack seeds and seedlings. The test also shows loss in seed germination and symptom development in seedlings and can be used to evaluate seed treatments (Mathur and Kongsdal, 2000).

The probable reason to explain why seed health testing is below standard is perhaps the shortage of testing equipment and trained seed pathologists (Mathur, 1983). At the University of Zimbabwe, seed pathology was recently introduced in the teaching programme at graduate level, meaning that the training, teaching and research in seed pathology at national level is far below satisfactory level. However, a number of people from the University of Zimbabwe, Seed Services and Plant Protection Research Institute have been trained at the Danish Government Institute of Seed Pathology for Developing Countries (DGISP) and can handle matters relating to seed health. Unfortunately, these seed pathologists go for greener pastures after course completion and the Seed Services Department is left deserted ([1]O. Randi (2004), personal communication). As a result, seed certification in the case of seed

[1] O. Randi is a former quality control expert at the Seed Services Institute and Seed Company of Zimbabwe.

health testing is not properly done leading to infested seeds being dispatched to farmers (Mcdonald and Copeland, 1998).

Another reason that might result in certified seed being infested by diseases is negligence by seed companies to follow proper certification procedures especially of the small grains such as sorghum in preference to the highly demanded and profitable larger grains of maize and soybeans. Multiple tests of certified and non-certified small grains seed lots by laboratory seed health testing procedures demonstrated that even the certified seed lots were infected considerably by smuts and bunts, indicating the need for laboratory testing with proper techniques (Mathur, 1983). Such weaknesses in the seed certification procedures in the seed industry significantly affect crop production. This justifies the adoption of this study on locally produced certified and farm saved sorghum seed and becomes a tool for formal seed suppliers to analyse the weaknesses in the seed production chain.

1.1 Objectives

The general objective of this study was to identify the fungal seed borne pathogens in farm saved and certified sorghum (*Sorghum bicolor*) seed and assess their effect on the crop growth and development in the field. The specific objectives were:

- To identify fungal seed borne pathogens in certified and farm saved sorghum seed samples.
- To determine the rate of seed to seedling transmission of selected fungal diseases.
- To evaluate the incidence and severity of seed borne and non-seed borne diseases in the field.

Therefore the hypotheses tested were:

- Fungal seed borne pathogens are present in both certified and farm saved sorghum seed.

- The transmission of some of these fungal pathogens from seed to seedlings is very high in sorghum.
- Seed borne diseases are prevalent with a high incidence and severity in the field.

CHAPTER 2

LITERATURE REVIEW

2.1 Sorghum production

Sorghum (*Sorghum bicolor* (L) Moench), referred to as the "poor man's" crop is the fifth most important cereal crop in the world and belongs to the family Poaceae (Gramineae). It is believed to originate in North East Africa where a large variability in wild and cultivated species is still found today and was probably domesticated in Ethiopia between 5000 and 7000 years ago (Arnon, 1972). Sorghum is among the leading cereal grains in Africa and is also important in India, China, Australia, various countries in Latin America and the United States of America. In Africa, where annual cereal production amounts to 89 million tonnes, sorghum is second in importance only to maize, and accounts for 14 % of the total cereal production, or 15,280 million tonnes per annum (CGIAR, 1997).

Although the developing countries of the semi-arid tropics account for 80 % of the total world area sown with sorghum, most of this is grown on a relatively small scale by smallholder farmers where it serves as a risk-reducing crop. In Zimbabwe, it is a crop of both the smallholder (mostly in natural regions III, IV and V) and commercial farmers in higher rainfall areas (Mtisi, 2002). It is reported that the area under sorghum during the 2003/04 season was 207,000 hectares, which is an increase of close to 300 % from 2002/03 season (UN Relief and Recovery Unit, 2004).

Sorghum is one of the most drought tolerant cereal crops and is usually grown in areas with climates too dry and hot for growing maize (CGIAR, 1997). This drought resistant characteristic of sorghum is due to its extensive root system as well as other morphological characteristics of its leaves and stalks that effectively reduce transpiration (Arnon, 1972). The crop has a high yield potential and the highest

recorded yield for the crop was about 20.1 tonnes per hectare depending on soil type and climatic conditions (Friis-Hansen, 1995). In Zimbabwe, historical average yield varies from 0.9 - 1.1tonnes per hectare giving an estimated total production in the range of 186 000 to 230 000 tonnes per annum (UN Relief and Recovery Unit, 2004).

2.2 Uses

Sorghum is produced primarily as a food grain throughout the world. It has varied uses that range from flour to local fermented and soured foods, porridges and beer. In the United States, sorghum is used primarily as a highly palatable feed grain for livestock. Its nutritional value is 90 % the equivalent of maize but it contains more protein and fat and is lower in Vitamin A and is deficient in the amino acid lysine (Mcdonald and Copeland, 1998). Industrial uses of sorghum include wallboard and biodegradable packaging materials (CGIAR, 1997).

2.3 Growing conditions

Sorghum can be established very successfully, under either conventional or reduced tillage systems, if the planting system provides good seed to soil contact and the surrounding soil is moist, and firm (Agrawal, 1995). It can be grown on a range of soils from light sands to heavy clays as long as they are in good tilth and will often produce a crop on land becoming too impoverished for many other cereals (Friis-Hansen, 1995). Soil pH readings of 5,0 to 5,5 (CaCl) that are slightly higher than those required for maize, are best for sorghum production (Seed Co., 2002). The crop is very tolerant of heat but very sensitive to cold and therefore minimum temperatures for germination are 7 ºC to 15 ºC. Optimum growth and yields are obtained when temperatures range between 25 - 35 ºC depending on the variety.

Sorghum, because of its fibrous root system, extracts soil nutrients from the soil very efficiently. Consequently, some farmers consider the crop as one that requires little or no fertilizer. However, grain

sorghum responds profitably to a balanced soil fertility programme. Recommended fertility levels vary with soil type, climate and yield goal (Seed Co., 2002).

2.4 Seed borne diseases of sorghum

Sorghum is plagued by a number of seed borne diseases of which the major ones are explained in detail below.

2.4.1 Sorghum wilt (*Acremonium strictum* W. Gams)

Acremonium wilt incited by *Acremonium strictum* has become an important disease of sorghum in Africa and Asia because of the cultivation of the recently developed high yielding cultivars (Chaudhary and Mathur, 1986). It has been recognised in many sorghum-growing regions including Egypt, United States of America and Mexico (Frederiksen, 1986). Infected seeds can be identified by visual examination and incubation tests. They are shrunken, desiccated, mummified, and misshapen and such seeds weigh less than healthy seeds and germinate poorly.

The fungus grows on incubated seed as a white mycelium that produces conidia. Colonies in the conidial state appear similar in colour and texture to some species of *Fusarium*. Conidia are hyaline, single-celled, and produced endogenously at the apex of the conidiophore in false heads. They are straight or slightly curved, and measure 2 - 5 x 1 - 1.5 µm. Perithecia are usually orange to red, ostiolate with a short neck, lined and contain several asci. Ascospores are pale brown to almost hyaline, single-celled, thick-walled with irregular, rough surfaces, and are 13 - 20 nm in diameter (Ahmed and Reddy, 1993).

The fungus can be controlled by the use of phytosanitary inspections, which aim to exclude the seed borne inoculum, by collecting seeds from disease-free plants. Information on seed treatment to eradicate

the seed borne inoculum of the fungus is not available. Breeding for resistant sorghum varieties has proved to be successful (Maude, 1996).

2.4.2 Grey leaf spot (*Cercospora sorghi* Ellis & Everhart)

Cercospora sorghi causes grey leaf spot of sorghum that is generally found in sorghum-growing areas where warm, wet weather prevails during the season. It is probably the most widely distributed foliar disease of sorghum that causes extensive foliar damage on susceptible cultivars. Disease progression and yield loss are influenced by initial inoculum potential, duration of environmental conditions favouring disease development and cultivar susceptibility (Frederiksen, 1986).

The fungus is present as dormant mycelia on and within infected seed, and can be detected by incubation tests. The fungus produces numerous light to dark grey conidiophores on the incubated seed. These conidiophores bear hyaline conidia which when massed together give infected seed a light greyish, velvety appearance. Conidiophores emerge from a stroma-like body on the surface of the seed, in small-scattered tufts of three to four, or more. They are medium to dark brown, narrower towards the apical tip, irregular in width, long with each conidiophore bearing one to six conidia (Zillinsky, 1982). The conidia are multiseptate (1 - 12 septa), hyaline, 30 - 300 x 2 - 4 μm, cylindrical, straight or slightly curved (Ahmed and Reddy, 1993).

The pathogen can be controlled by conducting pre-export crop health inspections during crop growth and selecting seeds from healthy non-infected fields. Fungicides such as benomyl, thiram, or chloranil can be applied at about 2.5 g active ingredient (a.i)/kg seed as seed dressings to eradicate seed borne infection (Ahmed and Reddy, 1993).

2.4.*3 Phoma sorghina* (Sacc.) Boerema, Dorenbosch, & van Kesteren

Phoma Sorghina causes leaf spot and blight of sorghum. In the field, the pathogen causes leaf lesions that measure approximately 5 x 2.5 mm. Lesions are generally parallel-sided with dark brown margins and light brown necrotic centres. Coalesced lesions usually result in tattered leaf tissue. Leaf margins are frequently necrotic. Pycnidia may form in the necrotic tissue (Zillinsky, 1982). Prolonged periods of continuous wetting are requisite for infection and symptom development (Wiese, 1977).

The seed borne inoculum of the fungus causes considerable damage. The fungus is carried as pycnidia and as dormant mycelium. Infected seeds can be detected by visual examination and incubation tests. Numerous pycnidia can be seen on dry seeds under a magnifying lens. The pycnidia are dark brown to black with the size of a pinhead and can be scattered throughout the surface of incubated seed (Mathur and Kongsdal, 2000; Mathur, Ram and Mathur, 1973). When the seed is heavily infected, the fungus can rupture the seed coat giving the seed a cankerous or warty appearance (Zillinsky, 1982). Sometimes fungal growth on incubated seed consists only of mycelium and chlamydospores. The mycelium is profuse, fluffy to dense, and is often very variable in colour. Sometimes pycnidia are produced on the aerial mycelium. Conidia are hyaline, single-celled, variable in shape and they measure 1.4 - 4.4 x 3.5 - 8.8 µm in diameter and are straight. Chlamydospores are frequently produced on the aerial mycelium and directly on the seed surface. They resemble *Alternaria spp.* spores, and are sometimes irregular in shape (Ahmed and Reddy, 1993).

Control of fungus includes selection of seed from noninfected plants combined with seed treatment. Seed treatment with thiram, captan or mancozeb (Dithane M-45) fungicides at about 3 g (a.i) / kg is advised to reduce the seed borne inoculum (Mathur and Manandhar, 1993).

2.4.4 Target leaf spot (*Bipolaris sorghicola* (Lefebvre & Sherwin) Alcorn)

The pathogen causes seed decay, seedling blight, leaf spot, and head mold of sorghum and pearl millet. Young plants and maturing plants are most susceptible to foliar blight. Seedling blight is more pronounced at temperatures of 25 ^{o}C and less. The disease is widely distributed and found in countries such as the United States, Hawaii, India, Japan, Zimbabwe and Zambia (Frederiksen, 1986).

The foliar symptoms vary from brown flecks, fine linear streaks, small oval spots, large irregular oval to almost rectangular spots measuring 1 - 10 x 0.5 - 3 mm depending on variety (Frederiksen, 1986). Lesions may expand and coalesce to form very long interveinal lesions. They may be solid dark brown but usually become tan or greyish brown with a more or less distinct dark brown border.

Infected seeds can be detected by incubation tests. The fungus grows on the incubated seeds producing mycelium, conidiophores, and conidia. Conidiophores are usually single or in small groups on the infected seed. The most distinguishing character of this species is that the primary conidia while still attached to the conidiophores frequently bear long secondary conidiophores on which small secondary conidia are produced (Ahmed and Reddy, 1993).

The pathogen can be controlled through seed treatment with ferbam at about 2.5 g (a.i) / kg seed (Almekinders and Louwaars, 1999).

2.4.5 Anthracnose (*Colletotrichum graminicola* (Cesati) G.W. Wilson)

Colletotrichum graminicola causes sorghum anthracnose that is one of the most important sorghum diseases limiting grain production worldwide (Vaillancoaurt and Hanau, 1991). The extent of damage or yield loss due to anthracnose is usually related to the degree of host susceptibility, the environment, the aggressiveness of the pathogen, and the physiological status of the host (Maude, 1996).

The disease is serious on sorghum, maize and rye. The leaf blight phase of the disease can limit production, with reductions in grain yield of 50 % or more in severe epidemics. Losses are greater when alternating wet and dry cycles occur with dry temperatures. The foliar phase of anthracnose results in small, elliptic to circular spots, usually 5mm or less in diameter. These spots develop small, circular, straw coloured centres with wide margins that are red, orange or tan depending on the cultivar (Frederiksen, 1986). The disease may defoliate sorghum plants and reduce growth and further development leading to plant death in severe cases. The fungus can overwinter on seed as dry acervuli and within the seed as a dormant mycelium. Seeds harvested from diseased plants are likely to carry the fungus (Wiese, 1977).

Infected seeds can be detected by visual examination and incubation tests. Dry seeds show visible symptoms of infection, in the form of dark brown to black acervuli scattered on their surface (Chaudhary and Mathur, 1986). These acervuli are irregular in shape and consist of dark setae. Sometimes acervuli are also formed on the glumes. On incubated seed, the fungus produces numerous acervuli, which are rounded or elongate, separate or confluent, superficial, erumpent, with conspicuous multicellular, darkly pigmented setae, and 70 - 300 μm in diameter. The acervuli consist of a gelatinous or mucoid, salmon orange coloured conidial mass. Conidiophores are hyaline, 8 - 20 μm long, and 4 - 8 μm broad. Individual conidia are hyaline, single-celled, spindle-shaped, with acute apices, and measure 19 - 28.9 x 3.3 - 4.8 μm. Setae are brown with a dark swollen base and a pale rounded tip (Ahmed and Reddy, 1993).

Control of the pathogen includes conducting pre-export crop health inspections during crop growth and discarding the mouldy seeds. Seed treatment with benomyl at about 2 g (a.i) / kg seed reduces the seed borne inoculum (Ahmed and Reddy, 1993).

2.4.6 Head blight and stalk rot of sorghum (*Fusarium moniliforme* J. Sheldon)

Fusarium moniliforme causes head blight, stalk rot of sorghum, and twisted top or top rot of pearl millet (Leslie, Pearson, Nelson and Toussoun, 1990; Wu and Mathur, 1987). Infected seed can be detected by visual examination and incubation tests. A white powdery fungal growth can be seen on dry infected sorghum seed. Sometimes, infected seeds of white-seeded sorghum cultivars have a pinkish or violet tinge (Ram, Neergaard and Mathur, 1970). Profusely infected seeds are reduced in size and weight, and do not germinate (Rheeder, Marasas and Van Wyk, 1990). The fungus usually produces a white to light orange powdery growth consisting of aggregated or loosely scattered chains of microconidia on incubated seed. Sometimes the microconidia may be produced on monophialides in false heads. The microconidia are hyaline, one to two-celled, 2 - 4 x 5 - 12 µm, and appear as beaded chains. They are oval to club-shaped with a flattened base. When the microconidia are not produced in chains, they might be confused with those of *F. oxysporum*. However, the phialides are longer and narrower in *F. moniliforme* than in *F. oxysporum* (Ahmed and Reddy, 1993).

Macroconidia are produced in pale orange sporodochia, which can be obscured by the mycelium and the abundant chains of microconidia. Macroconidia are produced on macroconidiophores. They are hyaline, 3 - 7 septate, 1.5 - 4 x 20 - 82 µm, slender, almost straight, and taper towards either end. They are slightly hooked at the tip, thin-walled, with the apical cell slightly curved and tapering to a point, and may be either distinctly or slightly foot-shaped at the basal cell (Frederiksen, 1986).

Control can be effected by discarding infected seeds and also seed treatment with carbendazim or a mixture of benomyl + thiram (Benlate-TIR), or carbendazim at about 2 g (a.i) / kg is advised (Ahmed and Reddy, 1993).

2.4.7 Sorghum smuts

Smuts are one of the most important diseases of sorghum especially where untreated seed is planted. Damage is confined almost entirely to the head or panicles, thus the reduction in yield is conspicuous and direct. The quality of the remaining yield is drastically reduced by the presence of the black smut spores on the surface of healthy kernels. Smut fungi seldom kill their hosts (biotrophs), but in some cases infected plants may be severely stunted (Agrios, 1997). Three sorghum smuts are common, the covered kernel smut, loose kernel smut, and head smut. Each one is caused by a different species of the fungus *Sphacelotheca*.

2.4.7.1 Covered Kernel Smut (*Sphacelotheca sorghi* (Link) G.P. Clinton)

Covered kernel smut, caused by the fungus *Sphacelotheca sorghi* attacks all groups of sorghums, including johnsongrass. Covered kernel smut is the most common disease of sorghum. Usually, all of the kernels in a smutted head are destroyed and replaced by dark brown, powdery masses of smut spores (teliospores or chlamydospores) covered with a tough, greyish white or brown membrane (Glair, Jenkins and Lester, 1991).

Its seed borne inoculum can be carried as admixtures of smut sori and as chlamydospores adhering to the seed surface. Smut sori are visible to the naked eye, and can be identified under a magnifying lens. Chlamydospores present on contaminated seed can be detected by a seed-washing test. Smut sori are oval, cylindrical, or nearly conical in shape, sometimes surrounded by unaltered glumes at the base. In some sorghum varieties, the shape of the smut sori is similar to that of normal grain, but when such grains are broken, they reveal the dark powdery spores of the fungus (Ahmed and Reddy, 1993). The chlamydospores of this fungus are very similar to those of *S. cruenta*, but are usually slightly smaller and somewhat paler brown (Zillinsky, 1982).

The pathogen can be controlled by avoiding seeds from endemic areas and discarding smut sori during visual examination. Apparently healthy-looking seed should be treated with carboxin (Vitavax) at about 2 g (a.i) / kg or elemental sulphur at about 5 g (a.i) / kg. If systemic seed dressings cannot be obtained soak the seeds in water for 4 hours, then dry them, first in the shade and then in the sun. This procedure kills germinating smut spores without impairing seed viability (Wiese, 1977).

2.4.7.2 Loose Kernel Smut (*S. cruenta*)

This is caused by the fungus *Sphacelotheca cruenta* and is less widespread than covered kernel smut. Loose kernel smut attacks all groups of sorghums although certain varieties in some groups are immune or highly resistant. Normally, all kernels in an infected panicle are smutted. Some kernels may be transformed into leafy structures or escape infection completely. Individual kernels are replaced by small smut galls (or sori) that are 2.5 cm or longer, pointed and surrounded by a thin gray membrane (Zillinsky, 1982). This membrane usually ruptures when or soon after the panicle emerges from the boot.

The sori produced can be detected in sorghum seed lots as admixtures during visual examination. In addition to smut sori, healthy-looking seed might also be contaminated by teliospores and chlamydospores adhering to its surface. Teliospores can be detected by a seed-washing test. Loose smut sori are visible to the naked eye and consist of masses of spore balls enclosed in a thick membrane, which splits irregularly from the apex downwards to reveal a dark, solid mass of teliospores. Teliospores are single-celled, brown and opaque, globose to oval or irregular in shape and 10 - 15 μm in diameter (Ahmed and Reddy, 1993).

Unlike covered kernel smut, plants affected with loose kernel smut are stunted, have thin stalks, and heads emerge earlier than healthy plants. Abundant side branches (tillers) also may develop.

Occasionally, the tillers are smutted, while the primary head is not. The fungus is heterothallic and is able to hybridize with both the covered kernel and head smut fungi, complicating the problem of developing resistant hybrids (Goates, 1988).

The pathogen can be controlled by conducting pre-export crop health inspections to select disease-free seed. Smut sori should be discarded during visual examination. Chemical seed dressing with carboxin at about 2.5 (a.i) g / kg which is a fungal respiratory inhibitor is effective in reducing the seed borne inoculum (Necombe and Thomas, 1990).

2.4.7.3 Head Smut (*Sphacelotheca reiliana* (Kohn) G.P. Clinton**)**

Head smut caused by *Sphacelotheca reiliana* is not so widespread and as damaging as the kernel smuts. Head smut attacks both maize and sorghums, being more common on the latter. Head smut has increased in severity proportionately to intensive cultivation of susceptible hybrids. Smutted plants also have weakened root systems and commonly exhibit more severe stalk and root rots than smut-free plants. Infection first appears when the young head, enclosed in the boot, is usually completely replaced by a large smut gall covered by a thick whitish membrane. The membrane soon ruptures, often before the head emerges, exposing a mass of dark brown to black, powdery teliospores intermingled with a network of long, thin, dark, broomlike filaments of vascular tissue (Bhale, Khare, Raut and Singh, 2001). The surfaces of seeds harvested from diseased fields are likely to be contaminated with chlamydospores of the fungus, which can be detected by a seed-washing test (Ahmed and Reddy, 1993).

Control of the pathogen can be effected by avoiding seeds from endemic fields. Seed treatment with carboxin at about 2.5 (a.i) g / kg or elemental sulphur at about 5 g (a.i) / kg helps to eradicate the seed borne inoculum (Glair *et al.*, 1991; Necombe and Thomas, 1990).

2.5 Economic significance of sorghum seed borne diseases

The practice of retaining seed and also poor certification procedures provide avenues for infection of seed by a number of diseases. Seeds carrying such diseases are detrimental to crop production because they may not germinate or may be of low vigour. This results in a decrease in the seedling population which leads to fewer adult plants with a concomitant reduction in crop yields. The infected seeds can also serve as sources of inoculum, which under suitable environmental conditions may introduce the disease into a healthy crop and thus reducing yields. The infected seeds carried into various geographical areas can introduce the pathogens in areas normally free of them. Seeds that are infected with fungi or bacteria, even if they have been treated with fungicide or bactericide, may still carry viable microorganisms that may cause the diseases (Agrawal, 1995; Basra, 2002). In severe infestations, pathogens are able to completely deform the seed, for example Acremonium wilt of sorghum and smuts of sorghum, reducing quality thereby making the seed unmarketable (Agrios, 1997).

2.6 Seed retention by smallholder farmers

Communal farmers do not have any tradition of purchasing improved open-pollinated sorghum and millet (Friis-Hansen, 1995). A survey carried out in Silobela, Zimbabwe in 1992 to assess the seed sources for smallholder farmers indicated that retention as seed source supplied an estimated 60 - 90% of the total seed use. In the case of sorghum, some farmers used a number of retained improved varieties, which were originally developed and released by Research and Specialist Services (R & SS). These included SV2, DC75, Red swazi and Chibuku (Friis-Hansen, 1995). They retained the seed till it declined in its performance. This decline in performance may be a result of continuous accumulation of seed borne pathogens with time leading to poor germination and crop stand hence reduced yields (Neergaard, 1979).

The practice of seed retention is popular as certified improved seed is expensive and there is also difficulty in accessing the seed since the formal industry has not organised multiplication and delivery of these small grains to the communal areas as it is not profitable. The improved seeds are open pollinated and farmers are thus able to retain the seed for years, before there is any need to purchase new seed. The market can therefore quickly be reduced to an economically unviable level (Friis-Hansen, 1995). Thirdly, the farmer who purchases the seed for the first time often expects it to perform better than the farm saved seed. If that expectation is not met, his confidence in improved seed may be totally destroyed (Mcdonald and Copeland, 1998). Low performance of such type of seed may be due to seed borne diseases and it is often released onto the market by the private seed companies without proper seed certification procedures followed. Although private enterprise participation in seed industry should be permitted and even encouraged, it should be tightly regulated to avoid contaminated or poor quality seed to be supplied to consumers (Mcdonald and Copeland, 1998).

2.7 Seed health testing

Seed health is a measure of freedom of seeds from pathogens. The presence or absence of seed-borne pathogens can be confirmed through the use of seed health testing (Agrawal, 1995). It includes visual examination of seeds externally or internally, macro or microscopically for the presence of pathogens as well as incubating seeds on agar or moist blotter papers and identifying the pathogens microscopically (Warham, Butler and Sutton, 1990).

2.7.1 Seed visual examination

Direct examination or inspection of dry seed is a qualitative and semi-quantitative seed health testing method where either the fruiting structures of fungi are detected under stereomicroscope or effects of fungal pathogens on the physical appearance of the seed are seen. For example in sorghum when normal seed is replaced by sori of spores of bunts and smuts or ergot (Mathur and Jorgensen, 1998). If seeds are

severely infected by some organisms they may be reduced in size or discoloured. For instance maize seeds infected with *Nigrospora* have white streaks with black spore masses near the tips and sorghum seeds infected with acremonium wilt are completely deformed (Agarwal and Sinclair, 1997). These contaminated seeds can be separated from the clean seeds thereby reducing percentage of infected seed in the seed lot.

2.7.2 Incubation tests

The seeds are incubated for a certain period in the agar plate or blotter test under specific environmental conditions in order to allow pathogens on the seed to grow. Different fungi are identified by features such as form, length and arrangement of conidiophores, the form, size, septation and chain formation of conidia.

Major factors promoting growth, fructification and development of symptoms of pathogens are temperature, humidity, light and period of incubation (Warham *et al.*, 1990).

2.7.2.1 Temperature

Temperature affects germination, growth and reproduction of organisms. It also affects growth of fungi and conditions for typical normal development are sometimes confined to a sharply defined range. In general practice, seeds are incubated on substrates at a constant temperature for example sorghum that is incubated at 20 (± 2) ºC, which favours growth of a large range of pathogens (ISTA, 1985).

2.7.2.2 Humidity

In the blotter test, the main problem is to keep the blotters adequately moist throughout the incubation period. An increase in the amount of water and number of layers of blotters per petri dish increases the

amount of moisture available. It is important that blotters be adequately moist, but too much water is detrimental to the test because it favours growth of contaminants (Warham *et al.*, 1990).

2.7.2.3 Light

This is important for the reproduction of the fungi. The extent of sporulation, spore morphology and pigmentation are influenced by light. In general, cool white fluorescent lamps or fluorescent black light lamps that emit radiation from near ultraviolet (NUV) to visible light (320 – 420 nm) are used. They give a much higher proportion NUV at 360 nm, which appears to be most suitable for routine seed health testing. This wavelength induces sporulation of a broad range of organisms without adverse effects (Mathur, 1983; Mathur and Manandhar, 1993).

Most fungi require NUV light and a subsequent period of darkness to complete sporulation and are known as diurnal sporulators. In these fungi, sporulation is divided into two distinct phases, an inductive phase and a terminal phase. The inductive phase results in the formation of conodiophores while the terminal phase result in the formation of conidia. Therefore, to induce sporulation of these diurnal sporulators, NUV light – darkness cycles of 12/12 hours are adopted (Warham *et al.*, 1990).

CHAPTER 3

MATERIALS AND METHODS

3.1 Collection of seed samples

A total of eighteen, 2kg farm saved seed samples were collected from Mbare Musika and these were from farmers of Mutoko and Murehwa. In addition to that, four, 5kg certified seed samples were obtained from Seed Co. and National Tested Seeds making a total of twenty-two different samples. These comprised seven red and fifteen white samples that were stored in a cold room at 5 ºC before testing to minimise deterioration.

3.2 Laboratory seed quality tests on sampled seed.

Sixteen samples were randomly chosen from the twenty-two seed samples and these were assigned accession numbers 1-16 and were further divided into four groups namely the white certified, the red certified, the white farm saved and the red farm saved samples. All the samples could not be used due to land limitation for the field trial.

3.2.1 Visual inspection of dry seed

The main purpose of this test was to examine dry seed for impurities, classified as inert matter in the International Seed Testing Association (ISTA) Manual, (1985) such as plant debris, and symptoms such as discolouration and malformation. The seeds were classified into three grades according to their size, discolouration, cracking and shrivelling using a seed appearance score as follows:

1 **Best**: Seeds of uniform size with less than 10 % shrivelling, discolouration or cracking on the seed coat.

2 **Good**: Seeds with between 10 – 50 % shrivelling, discolouration or crinkling and had variable size.

3 **Bad**: Seeds with greater than 50 % discolouration, crinkling or cracking of the seed coat.

The contents in these different categories were weighed and recorded.

3.2.2 Seed germination tests

The aim of the germination test was to determine the maximum germination potential of a seed lot, which in turn was used to compare the quality of different seed lots and also to estimate their planting value.

Germination tests were performed on the original samples and the selected categories. Twenty-five seeds were plated on wet blotter papers laid in petri dishes and each sample was replicated four times. These were incubated under darkness at 25 ºC for a period of 7 days. Recording started on the 4^{th} day of incubation as recommended by the ISTA (1985). Recordings were made on total germinated seeds, normal seedlings (seedlings that show the potential to grow into normal plants under favourable conditions), abnormal seedlings (seedlings not showing potential to grow into normal plants), ungerminated fresh seeds (dormant seeds that are hard and fresh), and ungerminated infected or dead seeds (seeds showing signs of pathogen infection or rotting) (ISTA, 1985).

3.2.3 Detection of seed borne fungal pathogens using the blotter test

Twenty-five seeds were randomly selected from a well-mixed working sample of 400 seeds from each of the sixteen samples using the hand halving method (Mathur and Kongsdal, 2000). The 25 seeds were plated equidistantly on petri dishes lined with 3 layers of water-soaked filter papers and each seed lot was replicated four times. The samples were then incubated for seven days under 12 hours alternating cycles of near ultra violet (NUV) light and darkness at 20 ^{o}C to stimulate fungal sporulation. The petri dishes were placed in a deep freezer after two days of incubation to kill the germinating seedlings. Recordings were made on working recording sheets for the blotter method with the aid of a low power stereomicroscope with X6.4 to X40 magnification to identify fungal habit characters.

Slides of fruiting structures mainly conidia were prepared and examined under the compound microscope at magnifications of X10 to X40 objective lens. Structures observed were compared to those in the Common Laboratory Seed Health Testing Manual for confirmation (Mathur and Kongsdal, 2000).

3.2.4 Seedling symptom tests

This method was performed to determine *Phoma sorghina* transmission from seed to the seedlings of sorghum. A required amount of 1% water agar was prepared and autoclaved. Approximately 15ml of water agar were poured into test tubes using an automatic dispenser and the test tubes autoclaved at 121 ^{o}C and pressure of 15 psi for 15 minutes. Agar was allowed to solidify by tilting the test tubes so as to get the required slant of the agar medium (approximately 30 ^{o} from the horizontal) to facilitate examination of fungi growing on ungerminated seeds under stereomicroscope. The seeds were surface sterilised in 0.5 % sodium hypochlorite for 2 - 5 minutes, rinsed in distilled water, blot dried and one seed dropped into each tube. The tubes were incubated under 12 hour alternating cycles of NUV light and darkness for at least 14 days. Each sample had a hundred test tubes.

Parts of the seedlings showing brown symptoms were further incubated in moist chambers for one week and examined. All seedlings with *Phoma* fruiting bodies were recorded. *Phoma* transmission efficiency was calculated for each sample by dividing *Phoma* infection percentage from the seedling symptom test by infection percentage from the blotter test.

3.3 Field trials

The aim was to check seed to field transmission of the pathogens. The 16 seed lots randomly selected from the 22 samples collected from Mbare Musika and seed companies were used in the field trials. These comprised 4 certified and 12 farmers' seed samples. One field experiment was conducted at the University of Zimbabwe's Department of Crop Science experimental plots under irrigation.

The field experiment was laid out in a randomised complete block design (RCBD) with 16 samples randomly allocated in 3 blocks, so that each accession appeared once in every block. The entire field area was (68 x 13) m^2. Each plot had 4 rows of sorghum 0,6 m apart and each row 3 metres long, with an area of 5.4 m^2. One-metre spacing was maintained between plots and 2 metres between blocks. The border rows consisted of the 2 outer rows and 50 cm on either of the remaining sides of the plot. Therefore, the net plot assessed was 2.4 m^2. Minimum tillage was practised and direct seeding was done mid-January. Gramoxone (24.75% Paraquat) herbicide was applied at the rate of 1.7 litres / hectare soon after planting to kill the existing weed population. Hoe weeding was done when necessary throughout the growing period. No disease control was implemented. Dimethoate (40% Dimethoate) and Dipterex granules (2.5% Trichlofon) insecticides were applied at 750 ml / hectare and 3 kg / hectare to control shoot flies (*Atherigona soccata*) and stalk borers (*Buseola fusca*) respectively.

Plant disease incidence and severity assessments were done fortnightly from the time symptoms started to develop for a period of 10 weeks. Disease incidence was determined by counting the number of

infected plants per net plot while disease severity was scored using 1 - 9 disease scoring scale where 1 represented 0 % infection and 9 represented over 85 % infection, developed by Ngwira and Pixley (1998). Hand harvesting of the panicles was done, placing them in different sacks according to their accessions. After drying and threshing, seed was weighed at 12.5 % moisture content.

3.4 Seed quality test on harvested seed samples.

Harvested seed was tested for the presence of fungal pathogens using the blotter test as described in section 3.2.3.

3.5 Data analysis

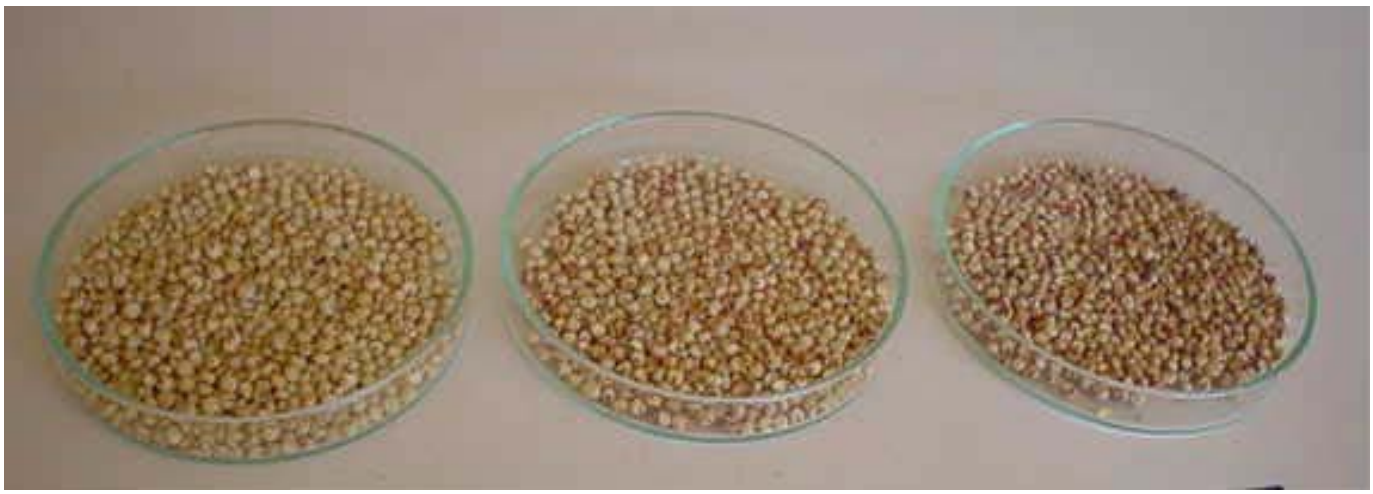
Disease severity data was used to calculate the area under the disease progress curve (AUDPC) using the trapezoidal integration program of Sigma Plot 2000 computer package. The AUDPC data were square root transformed and percentage incidence for the seed borne pathogens were arc sin transformed and then analysed using Minitab Statistical Package to generate ANOVA tables. Mean separation was performed by Fishers' Least Significant Difference (LSD) using MSTATC statistical programme. A homogeneity test was carried out for pathogen incidence of original and harvested samples to determine if they could be combined and were found to be heterogeneous.

CHAPTER 4

RESULTS

4. 1 Inspection of dry seed samples

The different seed grades used in the experiment are shown in fig 1.



1 2 3

Fig 4.2: Three petri dishes with the three sorghum seed grades
1 represents best grade – 10 % shrivelling, discolouration and cracking of seed coat
2 represents good grade – 10 – 50 % shrivelling, discolouration and cracking
3 represents bad grade – more than 50 % shrivelling, discolouration and cracking

The mean percentage seed appearance score that was found by selecting seed samples into different categories is presented in Figure 4. 2.

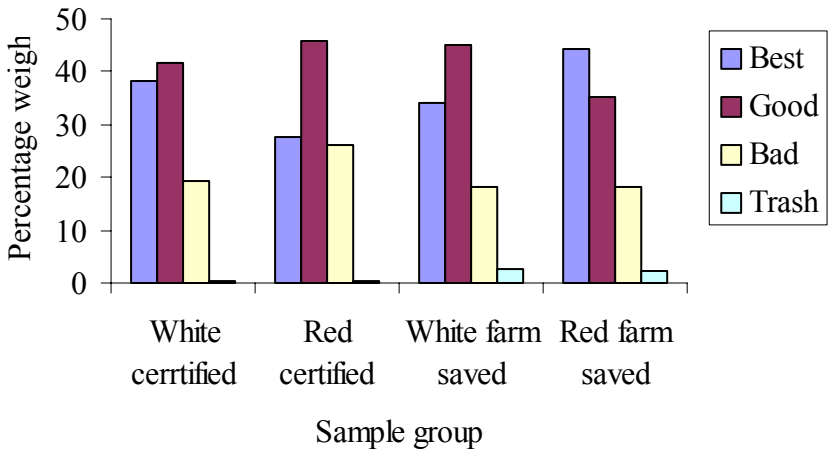


Fig 4.2: Mean percentage seed weight appearance for the sample categories

The seed appearance score shows that the red farm saved samples had the highest mean percentage seed weight of 44.4 % in the best grade followed by the white certified and white farm saved samples with mean percentage seed weights of 38.2 % and 34.1 % respectively. The red certified samples recorded the highest mean percentage seed weight (26.3 %) in the bad grade followed by the white certified samples while the white farm saved samples contained the highest mean percentage trash weight of 2.5 %.

4.2. Seed germination tests

Results for seed germination tests are presented in Table 4.1. The data was normally distributed hence not transformed.

Table 4.1: Mean percentage germination for the original sample groups before selection.

	Seedlings		Ungerminated seeds	
Sample group	Normal	Abnormal	Infected	Uninfected
White certified	11.50 a	7.88	4.00	2.75 a
Red certified	9.25 c	8.25	5.13	2.50 a
White farm saved	13.15 a	7.75	3.48	0.70 b
Red farm saved	11.00 ab	7.00	6.63	0.25 c
LSD	**1.930**	**N S**	**N S**	**0.673**

NB: Means followed by different letters in a column are significantly different at $P < 0.05$.

There were statistically significant differences among samples for normal seedlings and ungerminated uninfected seeds and no statistically significant differences for abnormal seedlings and ungerminated infected seeds. The white farm saved samples had the highest mean percentage normal seedlings of 13.15 followed by the white certified samples with a mean value of 11.5 %. The red certified samples had the least normal seedlings. The white certified and red certified samples had high mean percentage ungerminated but clean seeds while the red farm saved samples had significantly low mean percentage undiseased seeds.

4.3 Identification of seed borne fungal pathogens – blotter test

The results of the pathogens identified on sorghum seeds in the blotter tests are shown in Table 4.2.

Table 4.2: Mean seed infection for pathogens detected on the seeds of the original samples.

Sample group	*Fusarium* spp	*Cercospora* spp	*P. sorghina*	*Bipolaris* spp	*Curvularia* spp	*Exserohilum*
White certified	2.75 b	8.38 ab	0.79 c (0.25)	0.79 b (0.88)	0.50 b	0.79 (0.00)
Red certified	2.13 b	4.75 b	0.79 c (0.88)	0.79 b (0.25)	1.00 ab	0.79 (0.00)
White farm saved	9.53 a	5.78 b	0.83 b (4.08)	0.84 a (5.75)	1.58 a	0.79 (0.25)
Red farm saved	9.38 a	11.00 a	0.87 a (8.50)	0.79 b (0.50)	0.63 b	0.79 (0.00)
LSD	**2.627**	**4.554**	**0.011**	**0.014**	**0.149**	**N S**
Infection range (%)	**8.5-38.1**	**19-33.5**	**1-34**	**1-3.8**	**2-6.3**	**0-0.1**

NB: Means followed by different letters in a column are significantly different at $P < 0.05$.
NB: Arc sin transformation was used and untransformed data is in brackets. Data for *Fusarium* spp., *Cercospora* spp. and *Curvularia* spp. was normally distributed and was not transformed.

Six fungal pathogenic genera were isolated from the different seed samples. These were *Fusarium* spp., *Cercospora* spp., *P. sorghina*, *Bipolaris* spp., *Curvularia* spp. and *Exserohilum* spp. All the sample groups were found infected by one or more of these seed borne pathogens. There were statistically significant differences among the sample groups ($P < 0.05$) for the seed borne pathogens detected except for *Exserohilum* spp. The farm saved samples had relatively higher infection levels of *Fusarium* spp. than the certified sample groups but relatively similar infection levels for the rest of the pathogens.

Seeds showing growth of *Fusarium* spp. did not germinate on the blotter and whenever germination occurred, the roots had brown discolouration. The certified samples however, had higher infection levels for *Cercospora* spp. than the rest of pathogens detected. *Exserohilum* spp. had the least infection percentage of all the pathogens.

4.4: The effect of seed selection on normal seed germination and incidence of seed borne pathogen

Results of seed selection effect on percentage normal seedlings, percentage incidence of *Fusarium* spp., *Cercospora* spp., *P. sorghina*, *Curvularia* spp. and *Bipolaris* spp. are shown in Table 4.3.

Table 4.3: The effect of visual seed selection on percentage normal germination and incidence of seed borne pathogens.

Factor	Normal germination	*Fusarium* spp	*Cercospora* spp	*P.sorghina*	*Curvularia* spp	*Bipolaris* spp
Main factor (sample group)						
White certified	45.7	11.5 b	20.5 b	(0.17) 0.79 c	(2.0) 0.81 b	(0.17) 0.79 b
Red certified	41.7	12.0 b	24.8 b	(1.17) 0.80 c	(4.3) 0.81 ab	(0.83) 0.79 b
White farm saved	35.7	33.0 a	26.6 b	(13.6) 0.92 b	(4.8) 0.83 a	(3.37) 0.82 a
Red farm saved	26.3	19.0 b	38.2 a	(18.8) 0.99 a	(2.7) 0.81 b	(1.83) 0.80 ab
LSD $_{(0.05)}$	**NS**	**8.125**	**10.370**	**0.0525**	**0.0206**	**0.0181**
Sub factor (category)						
Best	47.7	19.1	25.0	(5.1) 0.84 b	(2.2) 0.81	(0.18) 0.79
Good	38.7	21.9	27.0	(7.5) 0.86 b	(2.3) 0.81	(1.90) 0.80
Bad	25.5	16.1	30.4	(12.9) 0.93 a	(4.8) 0.81	(1.58) 0.80
LSD $_{(0.05)}$	**NS**	**NS**	**NS**	**0.0525**	**NS**	**NS**
Sample group * Category	NS	NS	NS	P = 0.000	P = 0.002	P = 0.005

NB: Means followed by different letters in a column are significantly different at $P < 0.05$.

NB: Arc sin transformation was used and untransformed data is in brackets. Data for percentage normal germination, *Fusarium* spp. and *Cercospora* spp. was normally distributed and was not transformed.

There was no statistically significant effect of seed selection on normal seed germination ($P > 0.05$). Seed selection also had no effect in eliminating seed borne inoculum of *Fusarium* spp. and *Cercospora* spp ($P > 0.05$) from the visually clean seed. However, its effect was highly significant ($P < 0.05$) in reducing inoculum for *P. sorghina*, *Curvularia* spp. and *Bipolaris* spp.

4.5 *P. sorghina* transmission efficiency – seedling symptom test

Results of *P. sorghina* transmission efficiency are shown in Table 4.4.

Table 4.4: *P. sorghina* transmission efficiency and comparison of *P. sorghina* incidence in original and harvested samples.

Sample group	Original sample % infection (Blotter test)	Seedling symptom test % infection	Transmission efficiency [1]	Harvested sample % infection (Blotter test)
White certified	11.5	1.0	8.7	0.0
Red certified	3.5	1.5	42.9	0.0
White farm saved	15.8	6.6	41.8	1.5
Red farm saved	34.0	8.5	25.0	0.5
Mean	**16.2**	**4.4**	**29.6**	**0.5**

[1] Transmission efficiency was calculated by dividing *P. sorghina* infection percentage from the seedling symptom test by infection percentage from the blotter test.

Transmission efficiency varied among the different sample groups. Red certified samples had the highest transmission efficiency of 42.9 % followed by the white farm saved samples with 41.8 % transmission efficiency. The white certified samples recorded the least transmission efficiency of 8.7 %. *P. sorghina* mean infection was lower in the harvested as compared to the original samples. The

certified samples had 0 % *P. soghina* infection in the harvested samples while the white farm saved and the red farm saved samples had 1.5 % and 0.5 % *P. sorghina* infection respectively.

4.6 Comparison of pathogen incidence in the original and harvested seed samples – blotter test

The results of pathogen incidence in original and harvested samples are shown in Table 4.5.

Table 4.5: Mean pathogen incidence for the pathogen on original and the harvested samples

		White certified	Red certified	White farm saved	Red farm saved	**LSD**
Original	*Fusarium* spp.	2.75 b	2.13 b	9.53 a	9.38 a	**2.627**
	Cercospora spp.	8.38 ab	4.75 b	5.78 b	11.00 a	**4.554**
	P. sorghina spp.	0.79 c	0.79 c	0.83 b	0.87 a	**0.011**
		(0.25)	(0.88)	(4.08)	(8.50)	
	Bipolaris spp.	0.79 b	0.79 b	0.84 a	0.79 b	**0.014**
		(0.88)	(0.25)	(5.75)	(0.50)	
	Curvularia spp.	0.50 b	1.00 ab	1.58 a	0.63 b	**0.617**
Harvested	*Fusarium* spp.	3.50 c	2.25 c	6.03 b	11.50 a	**2.173**
	Cercospora spp.	0.82 a	0.83 a	0.80 b	0.79 b	**0.010**
		(3.50)	(4.00)	(1.28)	(0.88)	
	P. sorghina	0.79 (0.00)	0.79 (0.00)	0.79 (0.38)	0.79 (0.13)	**N S**
	Bipolaris spp	0.79 (0.00)	0.79 (0.00)	0.80 0.20)	0.79 (0.50)	**N S**
	Curvularia spp.	0.79 (0.00)	0.79 (0.00)	0.79 (0.10)	0.79 (0.13)	**N S**

NB: Means followed by different letters in a row are significantly different at $P < 0.05$.
NB: Arc sin transformation was used and untransformed data is in brackets. Data without transformation was normally distributed.

The results show that the original samples had higher disease incidence than the harvested samples. For instance, mean *Cercospora* spp. infection was higher for the original samples than the harvested samples for all sample groups. There were statistically significant differences among sample groups ($P < 0.05$) for *P. sorghina*, *Bipolaris* spp. and *Curvularia* spp. for the original samples but no differences ($P > 0.05$) for the harvested samples since mean infection was very low for all the sample groups. All the five fungal pathogenic genera were present in both the original and the harvested samples of the white farm saved group but with lower infection levels in the harvested samples.

4.7: Field trial results

The diseases observed in the field trials were anthracnose (*C. graminicola*), grey leaf spot (*C. sorghi*), leaf blight (*P. sorghina*) and covered kernel smut (*S. sorghi*) and these are shown in Figures 4.3 - 4.6.

Fig 4.3: Sorghum leaves showing symptoms of anthracnose (*Colletotrichum graminicola*) – arrows indicate the lesions

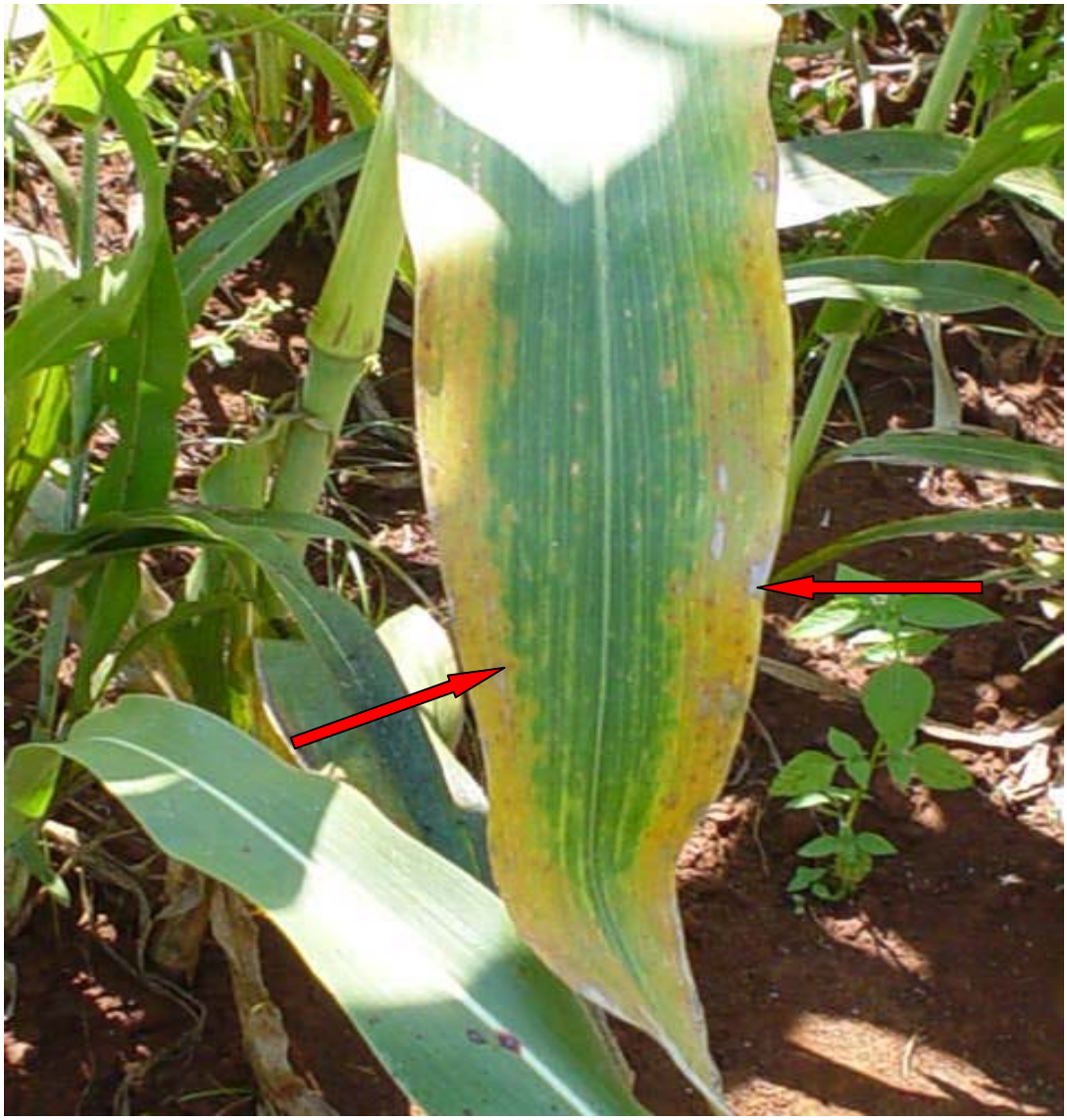


Fig 4.4: Sorghum leaves with grey leaf spot (*Cercospora sorghi*) – arrows indicate the lesions

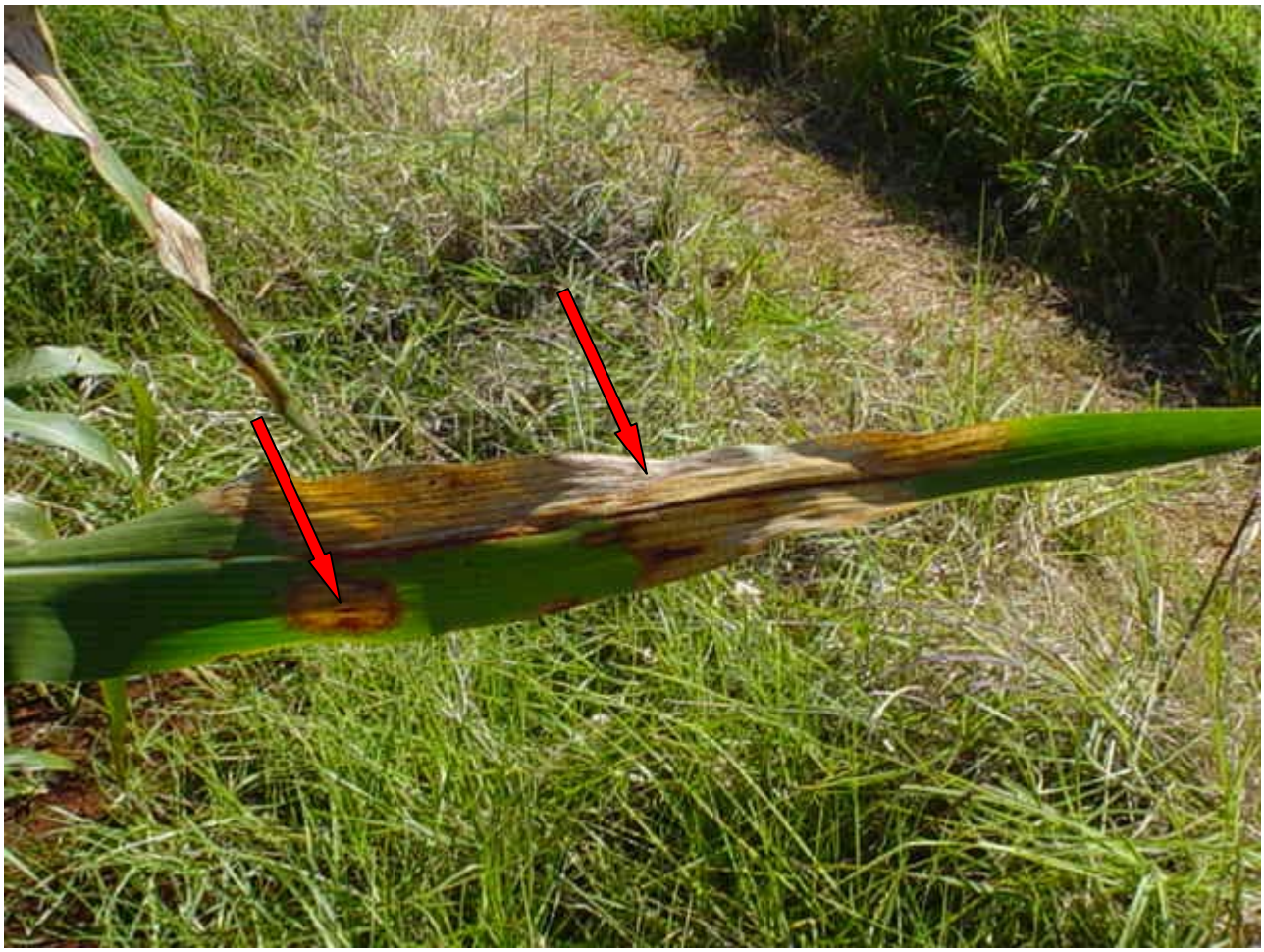


Fig 4.5: Sorghum leaves infected by leaf blight caused by *Phoma sorghina* – arrows indicate the blight symptoms.

Fig 4.6: Sorghum head with covered kernel smut (*Sphacelotheca sorghi*) – arrow indicates the smut sori.

The results of the mean area under disease progress curve (AUDPC) and yield for the different seed sample groups are given in Table 4.5.

Table 4.5: The mean area under disease progress curve and yield for the samples.

Sample group	AUDPC for diseases observed			Yield (tonnes/ha)
	Anthracnose	Grey leaf spot	Leaf blight	
White certified	3.06 (14.40) c	6.31(39.30) a	0.71(0.00) d	2.80 a
Red certified	6.88 (47.10) a	0.71(0.00) c	3.86 (15.43) b	2.81 a
White farm saved	5.26 (32.43) b	3.40 (16.63) b	1.74 (3.30) c	1.30 b
Red farm saved	6.55 (43.03) a	0.71 (0.00) c	4.95 (24.87) a	0.80 b
LSD	**0.789**	**0.303**	**0.571**	**1.225**

NB: Means followed by different letters in a column are significantly different at $P < 0.05$.
NB: Square root transformation was used and the untransformed data is in brackets. Yield data was normally distributed and was not transformed.

There were statistically significant differences among the different sample groups ($P < 0.05$) in the diseases and yields recorded.

For anthracnose, the red samples (both certified and farm saved) showed the highest area under disease progress curves followed by the white farm saved and lastly the white certified samples. For grey leaf spot, white certified samples had the highest AUDPC followed by the white farm saved samples. The red samples had the lowest AUDPC for grey leaf spot. The red farm saved samples had the highest leaf blight severity followed by the red certified samples. The white samples had the least AUDPC for leaf blight. Covered kernel smut was observed in the white farm saved samples only with mean infection of

8.1 %. There were statistically significant differences among accession groups in terms of yield ($P < 0.05$). The certified samples had higher mean yield than the farm saved samples.

CHAPTER 5

DISCUSSION

The content of pure seed is regarded as the proportion by weight of the sample that consists of structures containing a caryopsis and a piece of seed more than half their original size (Wellving, 1984). In this study, the seed appearance scores showed no quality differences in terms of seed size, shriveling and discoloration between the certified and farm saved seed. Both sample types scores were found in the best and bad grades, for example, the red farm saved samples scored highest mean percentage weight in the best grade while the white certified samples also scored high in the same grade. On the other hand, the red certified samples had the highest mean percentage seed weight in the bad grade while the red farm saved samples scored lowest mean weight percentages in the same grade. These results showed that both sample types fell within the same quality range. This could also be an indication that seed companies just follow the normal agronomic procedures for sorghum seed production.

Generally, germination test results showed a better percentage normal germination for the farm saved samples as compared to the certified seed lots. The white farm saved samples scored the highest normal seedlings while the red certified seed samples showed lowest normal seedling percentages. Probably, the certified seed was saved for a few seasons. The two certified sample groups had highest percentage ungerminated non-infected seeds. This could be due to the fact that the surface seed treatment used for certified seed suppressed fungal growth on the ungerminated seeds, which could have been dead seeds.

Inspection of dry seeds and its subsequent selection into different grades according to shrivelling and discolouration has been shown to have positive impact on seed health and this is closely associated with purity analysis as practiced in seed testing and quarantine stations (Mathur and Kongsdal, 2000).

Warham *et al*., (1990) confirmed that if seeds are severely infected by some seed borne pathogens, they become discoloured, for instance wheat seeds severely infected with *Fusarium* spp. are shrivelled and have a pinkish colour. Moreover, sorghum seeds infected with *C. graminicola* have dark streaks and spots on the seed coat (Mathur and Kongsdal, 2000). In this study, seed selection had a significant effect in reducing pathogens such as *P. sorghina*, *Curvularia* spp. and *Bipolaris* spp. No significant effect was shown in reducing *Fusarium* spp and *Cercospora* probably because the selection criterion used was not effective in eliminating inoculum for these pathogens from visually clean seed.

The blotter tests detected six genera of pathogenic fungi on almost all the samples. These were *Fusarium* spp, *Cercospora* spp, *Phoma sorghina*, *Bipolaris* spp, *Curvularia* spp and *Exserohilum* spp. and all these pathogens were reported to be seed borne on sorghum from literature. The level of seed borne infection with these fungi varied among seed lots. Agrawal and Sinclair (1997), also reported variable levels of seed borne infections among seed lots. These results confirmed the presence of seed borne pathogens on both farm saved and certified sorghum seeds. This is in agreement with findings of Manyangarirwa and Leth (2002) who also detected seed borne fungi on certified and non-certified sorghum seed lots.

The *Fusarium* spp that occur on sorghum include *F. moniliforme*, *F. fusarioides*, *F. oxysporum* and *F. semitectum*. In this study, *Fusarium* spp were found to reduce germination on blotters by 70 – 80 % and the roots of infected seedlings showed brown discolouration. This is in agreement with work done by Mathur, Ram and Mathur (1973), who reported high incidence of *F. moniliforme* embryonal infection in the seed and the embryos showed signs of rotting and this could be the cause of seed rots. In another study carried out by Mathur, Mathur and Neergaard (1975), root and shoot lengths of seedlings grown in pots were greatly affected by *F. moniliforme* with the roots showing pronounced browning and the shoots appearing highly blighted.

Bipolaris spp detected on seed samples in this study could be *Bipolaris maydis* and *B. sorokiniana* that have been known to be seed borne on sorghum and pearl millet. However, there are occasional references of these pathogen species on sorghum seed but none pertaining to their significance in the field (Fatima, Mathur and Neergaard, 1974). Mathur *et al*. (1975) found *B. maydis* on maize therefore, testing of sorghum for this pathogen is of utmost importance to enable precautions against spread of inoculum to maize fields.

Curvularia lunata has been recorded by Benoit and Mathur (1970), to be seed borne on sorghum and could be the species detected on seeds in this study. Germination failure of infected seeds confirms work done by Mathur *et al*. (1973), where seeds inoculated with *C. lunata* were sown in soil and showed considerable reduction in germination and the seedlings exhibited brown disclouration of the coleoptiles.

Seed selection has been reported to reduce pathogen infection levels in seed lots (Mathur and Kongsdal, 2000). However, in this study, for *Fusarium* spp and *P. sorghina*, seed selection was not effective in reducing inoculum levels in the best grade category. This could probably mean that the seed selection criterion used was not the best for eliminating infected seeds with these pathogens from the clean seeds of sorghum. Agarwal and Verma (1983), also reported that visual selection could not be effectively used for seeds with slight pathogen infections and seeds treated with chemicals. However, for other pathogens like *Cercospora* spp, *Bipolaris* spp and *Curvularia* spp, seed selection resulted in a reduction of inoculum levels in the best grade category. The sample groups habouring these pathogens had an increasing percentage of infected seeds from the best to the bad categories.

The seedling symptom test carried out in the study to detect *P. sorghina* transmission revealed high transmission efficiency of the disease from the seed to the seedlings but not to the grain. For instance the

red certified samples that had 42.9 % mean *P. sorghina* transmission efficiency had 0 % infection *P. sorghina* in the harvested samples. This was also observed for all the other pathogens detected. There was poor transmission of the seed borne pathogens recorded from the original samples to the harvested samples. This result showed the non-systemic nature of these pathogens. Unlike the sorghum smuts that appear on the grain, these pathogens can be transmitted from the seed to the seedlings but for them to infect the seed of these seedlings, they have to infect the inflorescence. Smuts are said to be systemic because they infect the emerging seedlings, grow internally in the seedlings until they reach the inflorescence, attack the ovaries and destroy grain kernels completely (Agrios, 1997; McGee, 1983). Several seed borne pathogens affect seed in the field during anthesis, seed development and seed maturation. *F. moniliforme* of maize has been shown to infect seed from seed development and the infection process continues during seed maturation (Castonguay and Couture, 1983). The infection process may however be influenced by environmental conditions for instance, rain and warm temperatures following anthesis resulted in increased grain mould contamination of sorghum caryopsis (McGee, 1995). In this study, these three growth stages occurred late in winter when there was reduced or no rainfall and low temperatures (Appendix 18). These conditions may have discouraged infection of the seed by the various seed borne pathogens and hence reduced pathogen spectrum in the harvested samples. Therefore, manipulation of planting dates in seed production as a strategy to avoid conditions favourable for seed infection can be used. The chances of temperature and humidity conditions favourable for infection occurring are much lower for late compared to early-planted crops (McGee, 1983; McGee, 1995).

On the other hand, the relationship of the pathogen to the seed may determine its success in infecting the harvested grain. Gabrielson (1988) noted that pathogens such as the smuts for example *Ustilago nuda* (Jens) Rostr. are carried within the embryo and a high proportion of infected seeds will develop into infected plants. Other pathogens that are carried with seed as dormant structures on or in seed coats may

just attack seedlings or rot infected seeds before the seedling reaches the soil surface, hence the presence of such pathogens cannot be indicated by seed health tests of the harvested grain.

There were significant differences among accessions for all the diseases observed in the field. The results showed anthracnose and leaf blight as the diseases prominent in the red samples. Grey leaf spot was prevalent in the white samples of which the white certified seed accessions had the highest mean AUDPC for grey leaf spot. The white farm saved samples had lower AUDPC for grey leaf spot. This could be a result of some white, tall, late maturing accessions in this group. Some studies have indicated that early maturing varieties are mostly attacked by diseases than late maturing cultivars (Ploper, Abney and Roy, 1992). Tall, late maturing varieties are typical of the local landraces that are well adapted to the prevailing conditions and can withstand local pathogenic infection unlike the early maturing improved varieties. Chaudhary and Mathur (1986), also mentioned that sorghum acremonium wilt had been shown to increase because of cultivation of the recently developed high yielding, early maturing varieties.

There were also significant differences among accessions in terms of yield. This might be a result of differences in genetic yield potential for the various sample groups. The results generally revealed that the certified samples were high yielding as compared to the farm saved samples. The way the experiment was designed could not cater for an assessment of the effect of disease on yield because the actual inherent yield potential for the accessions used was not known. However, the white samples that were attacked by covered kernel smut suffered yield loss in terms of quality. Even if quantitative yield loss was minor, smut contamination of the grain was sufficient to reduce quality and for smuts, disease incidence is equated directly to yield loss (Copeland and Mcdonald, 1995).

In the study, no disease severity was assessed for covered kernel smut because the disease attacks individual kernels before the head emerges and it rarely spreads to healthy kernels. Teliospore contamination of the grain in the field or during threshing will infect the seedling during seed germination (Ahmed and Reddy, 1993). The disease affected the white farm saved accessions only since the seed borne inoculum can be effectively controlled by appropriate chemical treatment such as carboxin (Vitavax), captan (Vitavax 300) and thiram (Vitavax 200) (Mathur and Manandhar, 1993). Therefore, the certified seeds were chemically treated and had no covered kernel smut. The disease was also not recorded in the red samples, maybe the red samples were resistant to the pathogen.

Most seed borne fungi detected on the seeds incubated on blotter paper (with the exception of *Cercospora* spp and *P. sorghina*) were not transmitted to crops in the field. Richardson (1989), stated that many seed pathogenic fungi are only of academic and quarantine interest since these agents usually do not cause a significant economic loss to the grower. This might mean that the pathogen levels found on seeds may be below levels required to incite a disease under field conditions for example one study by Gabrielson (1988), *Phoma lingam* that causes blackleg of Brussels sprout, reported no disease in the field unless seed infection levels were above 0.6 %. Therefore, there is need to establish inoculum threshold levels for the different seed borne pathogens which is an important aspect when clean seed is used as a disease control measure (Kuan, 1988; Nameth, 1998, Geng , Campel, Carter and Hills, 1983). However, in spite of its importance, inoculum threshold is difficult to establish since it is influenced by such factors as the environment, cultural and agricultural practices and certification or quarantine requirements (Schaad, 1988). For example, the threshold level must be zero for a disease protected by quarantine but however, for management purposes some infection percentage above zero predicting no effect must be established based on field experience (Stace-Smith and Hamilton, 1988). Generally, it is stressed that the threshold level be determined by correlation between seed infection level established by seed testing and field disease damage data established by well designed experiments (Russell, 1988).

On the other hand, *C. graminicola* that was not isolated from the seed was observed in the field much earlier than most of the diseases recorded. Although previous studies by Basu Chaudhary and Mathur (1979) confirmed that the pathogen is seed transmitted in sorghum and maize, it is possible that the disease might have originated from sources other than seed. It has been reported that the pathogen can persist in soil, infected crop residues and on weed hosts (Agrios, 1997). The origin of the pathogen from the crop residues might be closer since the sorghum was planted using minimum tillage on land where maize was previously grown. *Cyperus* species, *Cynodon dactylon, Eleucine indica* and *Rottboellia conchinchinensis* that were in and surrounding the plot could also be potential sources of inoculum.

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The farm saved and certified sorghum seed is highly infested with a number of fungal seed borne pathogens and this can affect germination capacity of the seed. Seed selection is useful in improving seed germination and reducing the seed borne spectrum of most pathogens of sorghum. Seed transmission varied among pathogens for example, *P. sorghina* and *Cercospora* spp that were detected in the laboratory, were also detected in the field, supporting the hypothesis that seed transmission of seed borne pathogens is high in sorghum. However, the other pathogens detected in seed were not transmitted in the field.

6.2 Recommendations

- Certification procedures should be improved in terms of seed health testing to ensure high quality seed with no seed borne inoculum.

- Farmers should be encouraged to practice seed selection as it reduces inoculum levels for some pathogens and also improves seed germination.

- Future research should focus on establishment of threshold levels for seed borne pathogens if seed health testing is to be effective in crop disease management. Establishment of pathogen tolerance levels for local areas coupled with accurate seed health tests, provide a useful disease management tool that can minimize the use of fungicides in crop production.

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APPENDICES

Appendix 1A: Analysis of Variance for normal seedlings

Source	DF	SS	MS	F	P
Replication	3	18.867	6.289	4.32	0.038
Treatment	3	27.268	9.089	6.24	0.014
Error	9	13.103	1.456		
Total	15	59.237			

Appendix 1B: Analysis of Variance for abnormal seedlings

Source	DF	SS	MS	F	P
Replication	3	67.607	22.536	7.89	0.007
Treatment	3	3.297	1.099	0.38	0.767
Error	9	25.721	2.858		
Total	15	96.624			

Appendix 1C: Analysis of Variance for ungerminated infected seeds

Source	DF	SS	MS	F	P
Replication	3	7.587	2.529	1.05	0.416
Treatment	3	23.327	7.776	3.23	0.075
Error	9	21.636	2.404		
Total	15	52.549			

Appendix 1D: Analysis of Variance for ungerminated uninfected seeds

Source	DF	SS	MS	F	P
Replication	3	1.5650	0.5217	2.94	0.091
Treatment	3	19.0200	6.3400	35.77	0.000
Error	9	1.5950	0.1772		
Total	15	22.1800			

Appendix 1E: Analysis of Variance for *Fusarium* spp. (Blotter test) – original samples

Source	DF	SS	MS	F	P
Replication	3	3.052	1.017	0.38	0.772
Treatment	3	197.527	65.842	24.41	0.000
Error	9	24.281	2.698		
Total	15	224.859			

Appendix 1F: Analysis of Variance for *Cercospora* spp. (Blotter test) – original samples

Source	DF	SS	MS	F	P
Replication	3	28.505	9.502	1.17	0.373
Treatment	3	94.205	31.402	3.87	0.050
Error	9	72.940	8.104		
Total	15	195.650			

Appendix 1G: Analysis of Variance for *P. sorghina* (Blotter test) – original samples

Source	DF	SS	MS	F	P
Replication	3	0.0001174	0.0000391	0.79	0.529
Treatment	3	0.0173204	0.0057735	116.70	0.000
Error	9	0.0004452	0.0000495		
Total	15	0.0178831			

Appendix 1H: Analysis of Variance for *Bipolaris* spp. (Blotter test) – original samples

Source	DF	SS	MS	F	P
Replication	3	0.0002072	0.0000691	0.93	0.466
Treatment	3	0.0082627	0.0027542	36.98	0.000
Error	9	0.0006703	0.0000745		
Total	15	0.0091402			

Appendix 1I: Analysis of Variance for *Curvularia* spp. (Blotter test) – original samples

Source	DF	SS	MS	F	P
Replication	3	1.6550	0.5517	3.71	0.055
Treatment	3	2.7950	0.9317	6.26	0.014
Error	9	1.3400	0.1489		
Total	15	5.7900			

Appendix 1J: Analysis of Variance for *Exserohilum* spp. (Blotter test) – original samples

Source	DF	SS	MS	F	P
Rep	3	0.05024	0.01675	1.00	0.436
Treatment	3	0.05024	0.01675	1.00	0.436
Error	9	0.15072	0.01675		
Total	15	0.25120			

Appendix 1K: Analysis of Variance for *Fusarium* spp. (Blotter test) – harvested samples

Source	DF	SS	MS	F	P
Replication	3	5.017	1.672	0.91	0.475
Treatment	3	201.727	67.242	36.46	0.000
Error	9	16.601	1.845		
Total	15	223.344			

Appendix 1L: Analysis of Variance for *Cercospora* spp. (Blotter test) – harvested samples

Source	DF	SS	MS	F	P
Replication	3	0.00010759	0.00003586	0.88	0.486
Treatment	3	0.00295271	0.00098424	24.20	0.000
Error	9	0.00036606	0.00004067		
Total	15	0.00342636			

Appendix 1M: Analysis of Variance for *P. sorghina* (Blotter test) – harvested samples

Source	DF	SS	MS	F	P
Replication	3	2.9003	9.6676	2.52	0.124
Treatment	3	3.7503	1.2501	3.26	0.073
Error	9	3.4503	3.8337		
Total	15	1.0101			

Appendix 1N: Analysis of Variance for *Bipolaris* spp. (Blotter test) – harvested samples

Source	DF	SS	MS	F	P
Replication	3	0.00003051	0.00001017	0.70	0.578
Treatment	3	0.00006702	0.00002234	1.53	0.273
Error	9	0.00013154	0.00001462		
Total	15	0.00022906			

Appendix 1O: Analysis of Variance for *Curvularia* spp. (Blotter test) – harvested samples

Source	DF	SS	MS	F	P
Replication	3	5.1877	1.7292	1.00	0.436
Treatment	3	5.1876	1.7292	1.00	0.436
Error	9	1.5563	1.7292		
Total	15	2.5938			

Appendix 1P: Analysis of Variance for Audpc for anthracnose (*Colletotrichum graminicola*)

Source	DF	SS	MS	F	P
Block	2	0.0493	0.0246	0.16	0.857
Treatment	3	27.0297	9.0099	57.78	0.000
Error	6	0.9356	0.1559		
Total	11	28.0146			

Appendix 1Q: Analysis of Variance for Audpc for grey leaf spot (*Cercospora sorghi*)

Source	DF	SS	MS	F	P
Block	2	0.084	0.042	1.86	0.235
Treatment	3	64.175	21.392	943.75	0.000
Error	6	0.136	0.023		
Total	11	64.395			

Appendix 1R: Analysis of Variance for Audpc for leaf blight (*Phoma sorghina*)

Source	DF	SS	MS	F	P
Block	2	0.0725	0.0362	0.44	0.661
Treatment	3	33.7534	11.2511	137.77	0.000
Error	6	0.4900	0.0817		
Total	11	34.3159			

Appendix 1S: Analysis of Variance for the yield

Source	DF	SS	MS	F	P
Block	2	16734	8367	0.39	0.695
Treatment	3	551588	183863	8.49	0.014
Error	6	129992	21665		
Total	11	698315			

Appendix 1T: Analysis of variance to show the effect of seed selection on *Fusarium* spp.

Source	DF	SS	MS	F	P
Sample group	3	3786.0	1262.0	39.31	0.000
Category	2	271.6	135.8	4.23	0.022
Interaction	6	432.3	72.1	2.24	0.061
Error	36	1155.8	32.1		
Total	47	5645.8			

Appendix 1U: Analysis of variance to show the effect of seed selection on *Cercospora* spp.

Source	DF	SS	MS	F	P
Sample group	3	2049.5	683.2	13.07	0.000
Category	2	238.7	119.3	2.28	0.116
Interaction	6	138.2	23.0	0.44	0.847
Error	36	1881.3	52.3		
Total	47	4307.7			

Appendix 1V: Analysis of variance to show the effect of seed selection on *P. sorghina*

Source	DF	SS	MS	F	P
Sample group	3	0.34693	0.11564	86.58	0.000
Category	2	0.06740	0.03370	25.23	0.000
Interaction	6	0.13697	0.02283	17.09	0.000
Error	36	0.04809	0.00134		
Total	47	0.59939			

Appendix 1W: Analysis of variance to show the effect of seed selection on *Curvularia* spp.

Source	DF	SS	MS	F	P
Sample group	3	0.005288	0.001763	8.50	0.000
Category	2	0.007025	0.003512	16.93	0.000
Interaction	6	0.005590	0.000932	4.49	0.002
Error	36	0.007468	0.000207		
Total	47	0.025371			

Appendix 1X: Analysis of variance to show the effect seed selection on *Bipolaris* species

Source	DF	SS	MS	F	P
Sample group	3	0.006990	0.002330	14.67	0.000
Category	2	0.000423	0.000212	1.33	0.277
Interaction	6	0.003583	0.000597	3.76	0.005
Error	36	0.005720	0.000159		
Total	47	0.016716			

Appendix 1Y: Analysis of variance to show the effect of seed selection on percentage normal seed germination

Source	DF	SS	MS	F	P
Sample group	3	2544	848	2.82	0.053
Category	2	4009	2004	6.66	0.054
Interaction	6	898	150	0.50	0.806
Error	36	10832	301		
Total	47	18283			

Appendix 2: Mean seed appearance score for the grouped samples (weight in grams)

Sample group	Sample category			
	Best	Good	Bad	Trash
White certified	38.2	41.8	19.2	0.45
Red certified	27.5	45.8	26.3	0.40
White farm saved	34.1	45.2	18.3	2.50
Red farm saved	44.4	35.2	18.2	2.10

Appendix 3: Raw data for normal seedlings for the grouped samples

Sample group	Rep 1	Rep 2	Rep 3	Rep 4
White certified	10.0	13.0	11.0	12.0
Red certified	8.0	10.5	7.5	11.0
White farm saved	12.4	13.4	13.3	13.5
Red farm saved	11.5	10.0	8.5	14.0

Appendix 4: Raw data for abnormal seedlings for the grouped samples

Sample group	Rep 1	Rep 2	Rep 3	Rep 4
White certified	5.5	9.0	7.0	10.0
Red certified	8.0	11.0	4.5	9.5
White farm saved	4.5	9.7	8.1	8.7
Red farm saved	2.5	11.5	6.0	8.0

Appendix 5: Raw data for ungerminated infected seeds for the sample groups

Sample group	Rep 1	Rep 2	Rep 3	Rep 4
White certified	4.5	5.0	5.0	1.5
Red certified	4.5	6.0	3.0	7.0
White farm saved	2.8	4.1	3.5	3.5
Red farm saved	6.5	8.0	4.0	8.0

Appendix 6: Raw data for ungerminated uninfected seeds for the sample groups

Sample group	Rep 1	Rep 2	Rep 3	Rep 4
White certified	3.5	3.5	2.0	2.0
Red certified	2.5	3.0	2.5	2.0
White farm saved	0.9	0.5	0.5	0.9
Red farm saved	0.5	0.5	0.0	0.0

Appendix 7: Mean infection for the original samples (Blotter test) - *Fusarium* spp.

Sample group	Rep 1	Rep 2	Rep 3	Rep 4
White certified	2.5	2.5	2.5	3.5
Red certified	1.5	0.5	4.5	2.0
White farm saved	9.9	10.0	9.8	8.4
Red farm saved	12.5	8.5	7.0	9.5

Appendix 8: Mean infection for the original samples (Blotter test) – *Cercospora* spp.

Sample group	Rep 1	Rep 2	Rep 3	Rep 4
White certified	10.5	15.0	4.5	3.5
Red certified	5.0	3.5	6.5	4.0
White farm saved	6.3	6.8	5.7	4.3
Red farm saved	11.5	11.5	9.0	12.0

Appendix 9: Mean infection for the original samples (Blotter test) – *P. sorghina*

Sample group	Rep 1	Rep 2	Rep 3	Rep 4
White certified	(1.0) 0.80	(0) 0.79	(0) 0.79	(0) 0.79
Red certified	(1.5) 0.80	(1.5) 0.80	(0) 0.79	(0.5) 0.79
White farm saved	(4.2) 0.83	(4.4) 0.83	(4.2) 0.83	(3.5) 0.82
Red farm saved	(7.5) 0.86	(9.0) 0.88	(9.5) 0.88	(8.0) 0.87

Appendix 10: Mean infection for the original samples (Blotter test) – *Bipolaris* spp.

Sample group	Rep 1	Rep 2	Rep 3	Rep 4
White certified	(1.0) 0.80	(1.5) 0.80	(0) 0.79	(1.0) 0.80
Red certified	(0) 0.79	(0) 0.79	(1.0) 0.80	(0) 0.79
White farm saved	(5.5) 0.84	(4.0) 0.83	(7.5) 0.86	(6.0) 0.85
Red farm saved	(0.5) 0.79	(0) 0.79	(1.0) 0.80	(0.5) 0.79

Appendix 11: Mean infection for the original samples (Blotter test) – *Curvularia* spp

Sample group	Rep 1	Rep 2	Rep 3	Rep 4
White certified	0.5	1.0	0.5	0
Red certified	0.5	1.5	1.00	1.0
White farm saved	2.2	1.8	1.1	1.2
Red farm saved	0.5	1.5	0	0.5

Appendix 12: Mean infection for the harvested samples (Blotter test) – *Fusarium* spp.

Sample group	Rep 1	Rep 2	Rep 3	Rep 4
White certified	5.5	3.5	2.5	2.5
Red certified	0.5	3.5	2.5	2.5
White farm saved	4.9	6.6	5.4	7.2
Red farm saved	10.5	13.5	12.0	10.0

Appendix 13: Mean infection for the harvested samples (Blotter test) – *Cercospora* spp.

Sample group	Rep 1	Rep 2	Rep 3	Rep 4
White certified	(4.0) 0.83	(3.0) 0.82	(2.5) 0.81	(4.5) 0.83
Red certified	(3.0) 0.82	(4.5) 0.83	(4.5) 0.83	(4.0) 0.83
White farm saved	(1.2) 0.80	(1.3) 0.80	(1.2) 0.80	(1.4) 0.80
Red farm saved	(0.5) 0.79	(0.5) 0.79	(1.0) 0.80	(1.5) 0.80

Appendix 14: Mean infection for the harvested samples (Blotter test) – *P. sorghina*

Sample group	Rep 1	Rep 2	Rep 3	Rep 4
White certified	(0) 0.79	(0) 0.79	(0) 0.79	(0) 0.79
Red certified	(0) 0.79	(0) 0.79	(0) 0.79	(0) 0.79
White farm saved	(0.9) 0.79	(0) 0.79	(0.4) 0.79	(0.2) 0.79
Red farm saved	(0.5) 0.79	(0) 0.79	(0) 0.79	(0) 0.79

Appendix 15: Mean infection for the harvested samples (Blotter test) – *Bipolaris* spp.

Sample group	Rep 1	Rep 2	Rep 3	Rep 4
White certified	(0) 0.79	(0) 0.79	(0) 0.79	(0) 0.79
Red certified	(0) 0.79	(0) 0.79	(0) 0.79	(0) 0.79
White farm saved	(0.1) 0.79	(0.5) 0.80	(0.1) 0.79	(0.1) 0.79
Red farm saved	(1.5) 0.80	(0) 0.79	(0) 0.79	(0.5) 0.80

Appendix 16: Mean infection for the harvested samples (Blotter test) – *Curvularia* spp.

Sample group	Rep 1	Rep 2	Rep 3	Rep 4
White certified	(0) 0.79	(0) 0.79	(0) 0.79	(0) 0.79
Red certified	(0) 0.79	(0) 0.79	(0) 0.79	(0) 0.79
White farm saved	(0.2) 0.79	(0.1) 0.79	(0) 0.79	(0.1) 0.79
Red farm saved	(0) 0.79	(0.5) 0.80	(0) 0.79	(0) 0.79

Appendix 17: Raw data to show the effect of seed selection on percentage normal germination

	Category											
	Best				Good				Bad			
	1	2	3	4	1	2	3	4	1	2	3	4
White certified	56	72	48	80	24	64	40	52	24	56	20	12
Red certified	40	56	64	36	20	48	36	56	44	28	48	24
White farm saved	20	68	52	44	44	36	72	12	16	32	20	12
Red farm saved	12	52	16	48	28	60	12	16	8	20	36	8

Appendix 18: Effect of seed selection on *Fusarium* spp. incidence on seeds – blotter test

	Category											
	Best				Good				Bad			
	1	2	3	4	1	2	3	4	1	2	3	4
White certified	8	16	4	6	18	8	12	16	4	20	14	12
Red certified	16	10	16	16	12	12	12	22	10	8	4	6
White farm saved	35.2	25.2	24.8	32	47.2	45.2	31.6	38	31.2	26.8	34.4	37.4
Red farm saved	30	24	20	22	4	18	24	30	16	10	20	10

Appendix 19: Effect of seed selection on *Cercospora* spp. incidence on seeds – blotter test

	Category											
	Best				Good				Bad			
	1	2	3	4	1	2	3	4	1	2	3	4
White certified	18	8	12	26	20	26	20	8	24	30	30	24
Red certified	22	22	20	26	22	16	38	26	18	18	42	28
White farm saved	25.2	28.8	25.6	23.2	27.6	25.6	22.8	23.2	19.6	37.2	30	30.4
Red farm saved	34	48	34	28	42	48	36	32	34	46	28	48

Appendix 20: Effect of seed selection on *P. sorghina* incidence on seeds – blotter test

	Category											
	Best				Good				Bad			
	1	2	3	4	1	2	3	4	1	2	3	4
White certified	0	0	0	0	0	0	0	0	2	0	0	0
Red certified	0	0	0	0	0	4	4	2	0	0	2	2
White farm saved	14.8	11.2	12.4	12.4	6.4	16	15.2	10.8	24.8	14	10.8	14.8
Red farm saved	10	6	8	6	20	14	10	16	32	30	42	32

Appendix 21: Effect of seed selection on *Curvularia* spp. incidence on seeds – blotter test

	Category											
	Best				Good				Bad			
	1	2	3	4	1	2	3	4	1	2	3	4
White certified	0	2	2	0	0	2	4	0	6	2	2	4
Red certified	2	2	2	0	4	2	0	4	6	4	4	4
White farm saved	0.4	2	3.2	4.8	4	2.4	3.2	3.2	8.4	6.8	10	9.2
Red farm saved	2	4	4	4	2	0	4	2	2	4	2	2

Appendix 22: Effect of seed selection on *Bipolaris* spp. incidence on seeds – blotter test

	Category											
	Best				Good				Bad			
	1	2	3	4	1	2	3	4	1	2	3	4
White certified	0	0	0	0	0	2	0	0	0	0	0	0
Red certified	0	0	0	0	0	2	0	2	0	2	2	2
White farm saved	1.6	5.6	0.8	0.8	1.6	2.4	4.8	3.6	5.2	3.6	4.8	5.6
Red farm saved	4	2	2	2	0	2	6	4	0	0	0	0

Appendix 23: Area under disease progress curves (AUDPC) for anthracnose (*C. graminicola*)

Sample group	Rep 1	Rep 2	Rep 3
White certified	(16.2) 3.23	(14.6) 3.08	(12.4) 2.87
Red certified	(53.4) 7.33	(40.6) 6.41	(47.3) 6.90
White farm saved	(31.8) 5.14	(34.3) 5.43	(31.2) 5.21
Red farm saved	(38.0) 6.16	(49.7) 7.08	(41.4) 6.42

Appendix 24: Area under disease progress curves (AUDPC) for grey leaf spot (*C. sorghi*)

Sample group	Rep 1	Rep 2	Rep 3
White certified	(36.4) 6.10	(43.8) 6.66	(37.7) 6.16
Red certified	(0) 0.71	(0) 0.71	(0) 0.71
White farm saved	(15.2) 3.26	(17.3) 3.29	(17.4) 3.44
Red farm saved	(0) 0.71	(0) 0.71	(0) 0.71

Appendix 25: Area under disease progress curves (AUDPC) for leaf blight (*P. sorghina*)

Sample group	Rep 1	Rep 2	Rep 3
White certified	(0) 0.71	(0) 0.71	(0) 0.71
Red certified	(15.2) 3.95	(17.2) 3.83	(13.9) 3.80
White farm saved	(3.4) 2.01	(3.2) 1.60	(3.3) 1.61
Red farm saved	(22.0) 4.62	29.6) 5.49	(23.0) 4.75

Appendix 26: Percentage incidence for covered kernel smut – field trial

Sample group	Rep 1	Rep 2	Rep 3
White certified	0	0	0
Red certified	0	0	0
White farm saved	6.5	8.3	9.6
Red farm saved	0	0	0

Appendix 27: Yield (kg / hactare) for the field trials

Sample group	Rep 1	Rep 2	Rep 3
White certified	727.50	805.45	485.91
Red certified	871.50	473.33	671.17
White farm saved	435.18	586.21	512.77
Red farm saved	282.88	510.65	484.35

Appendix 28: Disease scoring system (Ngwira and Pixley, 1998)

Percentage diseased tissue	Score (1 - 9)
0 – 2	1
3 – 5	2
6 – 10	3
11 – 25	4
26 – 44	5
45 – 60	6
61 – 75	7
76 – 80	8
81 – 100	9

Appendix 29: Average monthly temperature and rainfall data for the year 2004

Month	Jan	Feb	Mar	April	May	June	July
Temperature (°C)	21.5	21.6	20.9	18.7	15.5	13.8	13.9
Rainfall (mm)	211.6	243.7	125.8	10.5	0	0	0