INCIDENCE OF SEED INFECTION IN FINGER MILLET AND SUNFLOWER COLLECTED FROM DODOMA, IRINGA AND MOROGORO REGIONS

BY

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A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN CROP SCIENCE OF SOKOINE UNIVERSITY OF AGRICULTURE.

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ABSTRACT

Surveys were conducted to assess the incidence of seed borne pathogens on fingermillet (*Eleusine coracana* (L.) Gaetrn) and sunflower (*Helianthus annus* L.) in seeds produced by farmers in Morogoro Rural, Kilosa districts (Morogoro Region), Kondoa, Dodoma Rural districts (Dodoma Region), and Njombe and Iringa Rural districts (Iringa Region), in Tanzania. A total of 72 seed samples were collected and taken to the laboratory for assay of seed-borne pathogens. The blotter method was used to detect fungal pathogens and identification was done based on colony morphology, characteristics of fruiting bodies and spores observed under the stereo and compound microscopes. Seed-borne bacterial pathogens were detected using the direct plating method and identified based on pigmentation, biochemical tests, carbon source utilization, and hypersensitive reaction (HR), as well as pathogenicity tests.

Three fungal pathogens (*Pyricularia grisea*, *Bipolaris setariae* and *B. nodulosa*) were detected in fingermillet seed samples. The level of seed infection ranged from 10 to 50%. *Pyricularia grisea* was more prevalent in most fingermillet seed samples tested. Only two fungal pathogens (*Altenaria altenata* and *Verticillium albo-artum*) were detected on most of the sunflower seed samples tested. Bacterial pathogens detected on fingermillet seed included *Pseudomonas eleusines*, *Xanthomonas campestris pv corocanae*, *Pseudomonas syringae pv syringae* and *P. andropogonis*. The pathogens *Erwinia caratovora subsp caratovora*, *P. marginalis pv marginalis*, *P. syringae pv tagetis*, *P. syringae pv helianthi* and *P. syringae pv aptata* were detected on sunflower seed samples. *Pseudomonas andropogonis* (causing bacterial stripe), was detected on fingermillet in samples collected from Ilonga Kilosa, Zanka Dodoma Rural district, Kinonko and Mikese Market in Morogoro Rural district. The result of this study has
also established very important information on the occurrence of fingermillet blast (P. grisea) and bacterial stripe (P. andropogonis) across the surveyed regions. This is the first report of P. andropogonis on fingermillet in Tanzania.
DECLARATION

I, Solomon David, do hereby declare to the Senate of Sokoine University of Agriculture that, this dissertation is my original work and that it has neither been nor currently being submitted for a degree award in any University.

Solomon, David                                                                       Date
(MSc. Crop Science)

The above declaration is confirmed

Prof. R. B. Mabagala                                                                       Date
(Supervisor)
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Also my heartfelt thanks to families of, Mr and Mrs Israel Mwanga; Mr and Mrs Mdami; Mr and Mrs Machange; Mr and Mrs Deo S. Temba; My friends, Geja, Glory Mbiha, Busungu, Yusuf and Cole for their moral support during the study period.
DEDICATION

This work is dedicated to my Mother, Sara J. Mganah, my Wife, Gladys G. Machange, our son Gyan, my Uncles Solomon and Reuben; Siblings, Eznath, Farida and Evelyne.
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<table>
<thead>
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<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>%</td>
<td>Percentage</td>
</tr>
<tr>
<td>AEZ</td>
<td>Agro-ecological zones</td>
</tr>
<tr>
<td>ASPS</td>
<td>Agriculture Sector Program Support</td>
</tr>
<tr>
<td>cfu</td>
<td>Colony Forming Unit</td>
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<tr>
<td>cm</td>
<td>Centimeter</td>
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<tr>
<td>Cm²</td>
<td>Centimeter square</td>
</tr>
<tr>
<td>CV</td>
<td>Coefficient of Variation</td>
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<tr>
<td>DFB</td>
<td>Deep Freezing Blotter</td>
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<tr>
<td>DMRT</td>
<td>Duncan’s Multiple Range Test</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
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<tr>
<td>e.g</td>
<td>Example</td>
</tr>
<tr>
<td>ECARSAM</td>
<td>Eastern and Central Africa Research on Sorghum and Finger millet</td>
</tr>
<tr>
<td>FAO</td>
<td>Food and Agriculture Organization</td>
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<tr>
<td>g</td>
<td>Gram</td>
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<tr>
<td>GC</td>
<td>Glycine-Cytosine</td>
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<td>Ha</td>
<td>Hectre</td>
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<tr>
<td>HR</td>
<td>Hypersensitive Reaction</td>
</tr>
<tr>
<td>ISTA</td>
<td>International Seed Testing Association</td>
</tr>
<tr>
<td>KB</td>
<td>King’s B Medium</td>
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<tr>
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<td>Kilogram</td>
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<tr>
<td>KOH</td>
<td>Potassium Hydroxide</td>
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<tr>
<td>LSD</td>
<td>Least Significant Difference</td>
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<tr>
<td>MATI</td>
<td>Ministry of Agriculture Training Institute</td>
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<tr>
<td>MC</td>
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<tr>
<td>pH</td>
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<tr>
<td>PHB</td>
<td>Phosphate buffer saline</td>
</tr>
<tr>
<td>rRNA</td>
<td>Ribosomal Ribonucleic Acid</td>
</tr>
<tr>
<td>SUA</td>
<td>Sokoine University of Agriculture</td>
</tr>
<tr>
<td>TARO</td>
<td>Tanzania Agricultural Research Organization</td>
</tr>
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</table>
CHAPTER ONE

1.0 INTRODUCTION

Seeds are an essential component of world trade and are distributed nationally and globally, for food or as seed. About 90% of all food crops in the world are propagated by seeds (Schwinn, 1994). They are also the passive carriers of pathogens, which are transmitted when the seeds are sown under suitable environmental conditions (Noble, 1957, 1971). Fungi, bacteria, viruses and nematodes can be disseminated through seeds. Many of the bacteria and fungi, which are seed-borne, cause seedling and plant diseases.

Fingermillet (*Eleusine corocana* (L.) *Gaetn*) is indigenous to East Africa and the region has tremendous diversity in finger millet. It is estimated to comprise about 8% of the area and 11% of production of all fingermillets in the world (Hulse et al., 1980). It is grown by smallholder farmers, Uganda (up to 500,000 ha) and Kenya (around 65,000 ha) are two of the main producers of finger millet. The crop is the only millet on large-scale production in Tanzania. The main growing areas are Rukwa and Mbeya regions in the Southern highlands of Tanzania and Mara region on the eastern side of Lake Victoria. It is also grown to a lesser extent in Kilimanjaro, Dodoma, Iringa and Ruvuma regions. The exact area under fingermillet in Tanzania is not precisely known as all government production records include finger millet, sorghum and other millet together (Ulvund and Mkindi, 1980).

Sunflower (*Helianthus annus* L.) is also widely grown under dry conditions in many parts of the world and is one of the important oilseed crops on a global scale (Villabolos et al., 1996). In Tanzania, sunflower is the major oilseed crop followed by
cottonseeds, groundnuts, sesame and soybeans (Madadi, 1998). Sunflower is a very important oilseed crop grown by the majority of small-scale farmers in the semi-arid areas of central Tanzania, which include Dodoma and Singida regions. Other producing regions include Morogoro, Rukwa, Iringa, Arusha and Shinyanga. The crop is ecologically suited for production in the country due to its drought tolerance nature as compared to other oilseed crops (TARO, 1987). In addition, it has high oil content, simple oil extraction technique, especially under small-scale farmer’s conditions.

Concern over the ease of distribution of seed borne pathogens by national and international trade in seeds has been expressed by pathologists over the years (Moore, 1946; Muskett, 1950; Noble, 1951; Neegaard, 1971). There is awareness that the increasing movement of seed germplasm around the world also provide an avenue for the dispersion of all plant pathogens (Hampton et al., 1982). Appreciation of risks caused by seed-borne pathogens requires the understanding of the life cycles and disease cycles of seed-borne pathogens and an understanding of the environmental factors, which influence disease development (Neegaard, 1981).

Despite the efforts by farmers to increase sunflower production by expanding the production area, sunflower yield has remained low to meet both local and national demands, leave alone export demand to the neighboring countries. Yield obtained by smallholder farmers are about 550kg/ha while those obtained from experimental plots may be over 2000kg\(^{-1}\) (TARO, 1987). The low sunflower yields in Tanzania are attributed to poor crop husbandry including the use of poor quality unimproved local varieties (Hedge and Havanagi, 1987; Sounda et al., 1983).
Several seed-borne pathogens of sunflower have been reported in Tanzania; *Altenaria helianthi* (Hansf.) Tubaki and Nishihara, causing leaf spot, (syn. *Helminthosporium helianthi* Hansf), *Macrophomina phaseolina* and *Septoria helianthi* (Ell. and Kell) and sunflower brown rot caused by *Pseudomonas solanacearum* (Clinton, 1960). Leaf spot and sunflower rust were the most important diseases at Mlingano Tanga (Allen, 1974). In 1980, a serious leaf blight outbreak caused by *Altenaria spp* was observed in three divisions of Iringa region (Bujulu, 1980).

Blast caused by *Magnaporthe grisea* is one of the serious constraints for finger millet production, causing yield reductions of up to 64% and 80% in Kenya and Uganda, respectively (Esele, 1989). During the 1997 needs assessment exercise in Uganda, farmers identified finger millet blast as one of the major constraints to millet production. The disease occurs throughout Uganda and in all other African and Asian countries where fingermillet is grown. Under field conditions the disease has been reported to cause less than 10 % yield loss (Emchebe, 1980). Other seed-borne finger millet diseases which have recently gained economic importance are *Cylindosporium* leaf spot which occurs 60 days after planting and progress towards maturity which impair the grain development; tar leaf spot (*Phyllachora eleusines*), virus like infections also occur especially on later planted finger millet, while *Helminthosporium* leaf spot, bacterial blight and *Sclerotium* wilt have been reported to be of minor importance in finger millet (Adipala and Mukiibi, 1985).

Information on the development of management strategies of seed-borne pathogens for both sunflower and fingermillet is currently lacking. However, availability of improved and clean seeds, are also major production constraints. This is because many farmers keep their own seeds, which normally are mixtures of local varieties for
the next growing season. Studies on the epidemiology of various seed-borne pathogens of sunflower and finger millet in Tanzania are still limited. Therefore, data generated from the proposed study will be very useful in identifying interventions/measures for management of seed-borne pathogens in finger millet and sunflower in the country and elsewhere.

1.1 Objectives

1.1.1 General objective

To evaluate seed-borne pathogens in Finger millet and Sunflower in Morogoro, Iringa and Dodoma regions in order to provide information for management of diseases caused by seed-borne pathogens.

1.1.2 Specific objectives

(i) To determine the extent of seed infection in farmer’s saved seeds.

(ii) To isolate and identify specific seed-borne pathogens on sunflower and finger millet.
CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Finger millet

2.1.1 Importance of the crop

Finger millet (*Eleusine corocana*), is a staple food crop in East Africa and is grown in a number of countries in the region. It is highly nutritious and contains high levels of minerals and protein (Hulse *et al.*, 1980). It is important for food security in the region, drought tolerant than maize and less susceptible to bird damage than sorghum. Moreover, its greatest advantage is that it can be stored for long periods without chemical treatment. Finger millet grain is higher in protein, fat and minerals than rice, corn or sorghum (Reed, 1976). It is usually converted into flour and made into cakes, puddings or porridge.

2.1.2 Finger millet seed-borne fungi

Smuts, ergot and leaf diseases have been reported on finger millet in all East African countries (Esele, 1995; Mansuetes, 1995). Anthracnose has also been reported in Burundi, Ethiopia, Kenya and Uganda. Leaf blight is a common disease in the highlands. Blast is the major disease affecting finger millet in the East African countries (ECARSAM, 2004). Some of the specific finger millet seed borne fungi include:

*Bipolaris nodulosa* (Berk and M. A. Curtis) Shoem, Syns *Helminthosporium nodulosum* Berk. and M.A. Curtis, *Dreschlera nodulosa* (Berk. and M.A. Curtis) Subram and Jain. The fungus causes seedling blight, foot rot and leaf blight diseases of finger millet. It produces two types of mycelia (Ranganathaiah and Mathur, 1978),
greyish to dark greyish mycelium with slender conidiophores and conidia covering a part of the seed or the whole seed. Often, the growth extends to the blotter. The other colony has scanty mycelium; conidiophores olivaceous brown, straight or flexuous, bearing dark brown conidia arranged acropleurogenously. Conidia are generally 5-9 septate, subcylindrical, slightly curved or straight, tapering towards the rounded ends, 55.5 – 87 * 14.5 * 21 µm.

*Bipolaris setariae* (Saw) Shoem. *Helminthosporium setariae* Saw.

*Drechslera setariae* (Saw.) Subram and Jain

This fungus causes zonate leaf blight; its growth which is characteristically greyish brown to black, covers usually the entire seed and spreads to the filter paper where sporulation can also be seen. Conidiophores are pale brown, straight or flexuos, medium to long, bearing 1 to 5 acropleurogenously arranged, pale to golden brown, slightly curved conidia. Conidia smooth – walled, fusoid, rarely straight, pale to mid golden brown, 5 to 10 distoseptate, 45 – 100 * 10 – 15 µm.

*Pyricularia grisea* Sacc.

The fungus causes blast of finger millet and generally produces greyish mycelium with conidiophores arising singly or in groups, covering part or whole of the infected seed. Conidiophores of the fungus are slender, grayish or pale brown, short, smooth, bearing clusters of conidia. Detection of *P. grisea* becomes difficult when *Bipolaris nodulosa* is also present as mixed infection on the finger millet seed. *Bipolaris nodulosa* often overgrows *P. grisea*. Conidia of *P. grisea* are typically obpyriform, hyaline, 2 septate, hilum protuberant and apex acute, 19 – 31 * 10 – 15 µm. The disease is also reported in Brazil and Southern Cone of Latin America. The yield loss
can be high due to lack of resistant varieties in hosts such as wheat, tritcale, rye, sorghum and maize.

2.1.3 Fingermillet: Seed borne bacterial pathogens, general characteristics and diseases symptoms

*Pseudomonas avenae* Manns, 1909.

The bacterium is Gram-negative rods, polar flagellate, non fluorescent, accumulates PHB; colonies on YDC medium are cream with tan to brown centers, convex, smooth, 2-3 mm diameter in 3 days. Old colonies are sticky and adherent to the agar. It does not fluoresce on KB medium, reduces nitrate but no denitrification; oxidase, arginine dihydrolase, potato soft rot are all negative. Hydrolysis of gelatin variable and starch slight. On its host the pathogen causes yellowish or brownish leaf streaks and stripes, which may extends into the sheaths. Occasionally a stalk rot develops. The host range include *Eleusine coracana*, *Echinochloa cruzgali*, *Sorghum bicolor*, and *Zea mays* (Shakya, 1983). The pathogen may occur in wetland and upland rice nurseries. It is widely distributed although seedling infection caused by the bacterium has only been reported from a limited number of countries (Fahy and Persely, 1983). The host range of the pathogen is also wide among the monocotyledonous plants including rice, oats, Italian millet and maize (Kuwajima, 1988).

*Pseudomonas cichorii* (Swingle) Stap 1928

A fluorescent rRNA Group I bacterium, this is distinct from the many that belong to *P. syringae*. It is a Gram-negative rod with a tuft of polar flagella; levan production on SNA, arginine dihydrolase and potato rot are negative, oxidase and tobacco HR
are positive. It does not produce pyocyanin or carotenoid pigments, hydrolyse starch or gelatin, reduce and denitrify nitrate (Lelliot et al., 1966). Leaf spots stem spots and rots are the results of the effects of this pathogen in wheat, finger millet, sesame and other vegetable and field crops. Browning and/or melanosis of stems of wheat have been reported. In Tanzania, the pathogen is known as *P. papaveris* (Piening and Macpherson, 1985). The pathogen was reported to attack geranium in the past few years, but due to exchange of plant materials, the pathogen is now the biggest threat to the flower industry in the Middle East and Africa (Moorman, 2005).

*Pseudomonas eleusines* (Billimoria and Hedge, 1971)

The bacterium is Gram-negative with 1-2 polar flagella, gelatin not liquefied, but starch hydrolysed; nitrate reduced; acid but no gas produced from glucose; weakly lipolytic; cause a systematic bacterial wilt of *Eleusine coracana* (ragi, family *Graminae*) Nishiyama et al., (1979).

*Pseudomonas andropogonis* (Smith) Stapp 1928.

It is Gram-negative rods, with 1, rarely 2 polar flagella. Frequently a sheathed flagellum is present (Fuerst and Hayward, 1969). Accumulates PHB as a cellular reserve; no fluorescent pigments produced. Negative results recorded for hydrolysis of gelatin and starch, nitrate reduction, lipolysis, arginine dihydrolase and usually oxidase. No pectolytic activity is shown on pectate gels or vegetable tissue. Catalase, urease, phosphatase and phenylalanine deaminase are positive. Acid is produced aerobically without gas and in small amounts from adonitol, arabinose, fructose, galactose, glucose, glycerol, mannitol, mannose, sorbitol and xylose, but not from cellubiose, dulcitol and glycogen. Some of this act as carbon sources while others are
not. The GC content of the DNA is 57.8 moles %. This is a heterogenous species with more than one serological group (50, 15) and Fuerst and Hayward (59, 55, 73) consider that the two original species attacking sorghum and velvet bean (Mucuna) “constitute specialized pathovars”. They did not, however, formally propose the names.

*Pseudomonas andropogonis*, as it stands at present, has a wide host range mainly in the two families, Graminae (*Sorghum alnum, Euchalaena mexicana, S.bicolor, S. chinense, Zea mays* and *S. technicum*) and Leguminosae such as *Trifolium repens, Vicia benghalensis, Phaseolus vulgaris, Lablab purpureuns, Medicago sativa* and *Vicia faba* are affected.

Leaf stripes in the Graminae and leaf spots in the legumes and occasionally stem rots are caused, especially in the young stems. Bacterial stripe is the most common disease also in fingermillet and is characterized by the formation of pigmented interveinal lesions which may reach 50 cm or more in length (Tarr, 1962), little is known with respect to the extent of damage caused by this disease or on effort to breed for resistance. A single isolate from sorghum produced an antimetabolite inhibitor of *Escherichia coli* B which was reversed by L-glutamine but not by other amino acid.


The pathogen causes bacterial blight of *E. coracana* and ragi or African millet. The disease attributed to *X. eleusine* is the same. Patel *et al.*, (1961) examined authentic
cultures of this latter organism and found that it was neither a *Xanthomonas*, nor pathogenic to *Eleusine*.

2.1.4 Management of finger millet diseases

The following management approaches have been recommended and may be effective in controlling finger millet diseases. They include crop rotation, appropriate planting dates, selecting disease-resistant varieties, cleaning and processing methods that control seed-borne pathogens. Seed treatment to reduce seed-borne fungi and bacteria especially the use of antagonists (bio-control), hot water treatment and seed disinfections are also recommended where appropriate.

2.2 Sunflower

2.2.1 History, geographical distribution and importance of sunflower

Sunflower (*Helianthus annus* L.) is one of the few crop species that probably originated from North America (Carter, 1978 and Putnam, 1990). It was introduced to Europe through Spain and Russia. Sunflower is now grown in most temperate and tropical areas (Purseglove *et al*., 1990). In Africa, sunflower is the major oilseed crop in Eastern and Southern parts of the continent (Acland, 1971; Raymond and Nalumansi, 1996).

Commercially, available sunflower varieties contain from 39 to 49 % oil content (Yadar and Harishankar, 1980 cited by Krishnamurthi and Marthan, 1996). Sunflower accounts for about 14 % of the world production of seed oils. The oil accounts for 80 % of the value of the sunflower crop. Non-dehulled or partly dehulled sunflower meal has been substituted successfully for soybean meal in ruminant animals. Sunflower is
also used in certain paints, varnishes, soap, detergents and plastics because of good semi-drying properties without color modification associated with oils high in linolenic acid.

2.2.2 Sunflower Seed-borne Fungi and Diseases they cause

The major diseases of sunflower include rust (*Puccinia helianthi*); downy mildew (*Plasmopara halstedii*), Verticillium wilt (*Sclerotinia sclerotiorum*), Sclerotinia stalk and head rot (*Verticillium dahliae*), Phoma black stem (*Phoma macdonaldii*) and leaf spot (*Septoria helianthi*). Resistance to rust, downy mildew, and *Verticillium* wilt has been incorporated into improved sunflower germplasm. The following are specific sunflower fungal pathogens.

*Altenaria alternata* (Fr.) Keissler. Syns. *Altenaria tenuis* Nees.

It is the causative pathogen of leaf spot disease of sunflower. Brown to black growth containing conidia in long chains are characteristics of the species. The conodial chains are usually simple, but can also be branched. Conidial beaks (between the conidia) can sometimes be seen at high magnifications. In most cases, growth of *A. alternata* covers the entire seed surface.

Conidia are polymorphous, short to long, olive brown to dark brown, highly variable in shape (oval to cylindrical), size and number of septation (transverse, longitudinal and oblique) 10 – 58 * 7 –19 µm (Neegard, 1945). Conidial beaks also show variation, short to long, mostly of the same color as the main body or a little lighter, 2 – 19 µm in length. Total length of conidia with beak 10 - 71µm. The fungus has a wide host range including florist’s geranium, dahlia hybrids, African daisy, African
violet and *Vinca* (Manulis, 1998). The disease is favoured by conditions that stress host plants such as high or low temperatures or closed boxes during shipping.


The fungus also causes leaf spot disease and it produces light grey to grey conidia on short conidiophores. The conidia appear translucent and growth appears olivaceous brown and the mycelium is mostly absent. The conidia are cylindrical to obclavate, with rounded ends, pale olivaceous brown or golden brown, smooth with 2 – 12 transverse septa, sometimes one or few longitudinal septa, prominent constrictions at the septa, 45 – 145 * 10 – 30 µm. Leaf spot disease can be serious in warm humid environments but their importance is usually minor. *A. altenata* is a common saprophyte and is often associated with declining plants, but its significance is unknown (Daughtrey *et al.*, 1995).

*Altenaria zinniae* Pape

The fungus causes leaf spot disease and its growth consists of conidia with long beaks in groups, sometimes single. Generally, no mycelium. At times the growth may spread to the blotter. Conidia mostly single, obclavate, pale to dark brown, 3 – 12 transverse septa, 0 – 9 longitudinal, few oblique, body 19.5 105 * 9 – 28.5 µm; beak filiform, simple 6 – 159 µm with 0 – 3 transverse septa. A secondary conidium may appear, but seldom. The pathogen is recognized to cause major effects in more humid areas of Europe, India, Australia and parts of Africa (Pearce, 2005). Yield losses may range from 15 to 90 percent, with oil losses of 20 to 30 percent.
**Botrytis cinerea** Pers. Ex Fr. Syns. *Haplaria grisea* Link. *Botrytis vulgaris* Link ex Fr.

The fungus causes grey mould and head rot of sunflower and it produces very long, brown conidiophores, which repeatedly branch and bear ashgrey to grayish brown clusters of conidia. Conidiophores show characteristic “twisting” at many places. Twisting is more apparent at high magnification of the stereomicroscope, eg *50.

Conidia ellipsoidal or obovoid, single-celled, often with a slight protuberant hilum, colourless to pale brown, smooth, 8 – 14 * 6 – 9 µm. This filamentous fungi are the principal pathogenic agents of plants and the damage provoked by these microbes is responsible for about 20 % of the crop losses in the world, and their cost is estimated at 10 – 100 billion euros per year (Leroux *et al.*, 2002).


**Botryodiplodia phaseoli** (Maubl.) Thirumalachar

*Macrophomina phaseolina* causes light to heavy growth of mycelia on seed, consisting of large pycnidia overgrowth with greyish hyphae, especially surrounding the neck of pycnidia. In a heavily infected seed the dark mycelium of the fungus covers the whole seed and the infected seed appears like a piece of charcoal. Sometimes, only the sclerotical stage of the fungus, *Sclerotium botanicola* Taub., consisting of sclerotia and dark mycelium might be seen on the seed. At times, both pycnidia and sclerotia are observed on the infected. Pycnidiospores of *M. phaseolina* are often seen oozing from ostiole of pycnidia in the form of cirrhus which is white and wet at the beginning, becomes drier with age. Spores are one-celled, hyline, ellipsoid to obovoid, thin walled, 14 – 30 * 5-10 µm. The pathogen infects over 500 plant species including important crops like sorghum, millets bean, cotton, soybean
and corn under favorable conditions and has a wide geographic distribution (Mihail et al., 1992).


Pycnidia from the stem canker disease can be found on seed, solitary or in groups. They have large ostioles and some pycnidia may be seen with wet ooze of pycnidiospores. Pycnidiospores in slimy masses can also be seen amongst closely placed pycnidia. Pycnidiospores are of two types, alpha and beta. Alpha are fusoid to ellipsoidal, biguttulate, rarely 3, 5-9 * 2.5 µm while beta are filiform, curved, rarely straight, 20-30 * 0.5 – 1 µm.

**Verticillium** spp

The genus is characterized by the presence of verticals of phialides on conidiophores, and colourless, small, circular, shiny watery drops at the tips of phialides in each verticil. The watery drops contain numerous conidia. Usually, the growth of the fungus is restricted and is close to seed surface. The arrangement of the verticillate phialides are usually erect on conidiophore. The identification of *Verticillium* to the species level in the blotter method based on the habit characters is impossible because of similarity in the appearance of shiny, watery drops on phialides. The situation becomes further difficult and complicated as more than one species can be found on the seeds of the same host.

For example, both *V. albo-artum* Reinke, Berthier and *V. dahliae* Kleb. Are known to be seed-borne in cotton, safflower and sunflower. *V. albo-atrum* and *V. dahliae* can
be identified when cultures of an agar are made from the watery drops. Resting mycelium is produced former species while the latter produces microsclerotia. Conidia arising singly at the apices of phialides in both species, they are ellipsoidal to irregularly sub-cylindrical, hyaline, simple, occasionally one septate. They measure 3.5-10.5 * 2-4 µm in *V. albo atra*um and 2.5 – 8 * 1.4 – 3.2 µm in *V. dahliae*. The pathogen has a restricted host range and geographic distribution and generally causes only mild diseases in hosts other than alfa alfa (Leath and Pennypacker, 1990).

2.2.3 Sunflower seed borne bacterial pathogens, general characteristics and diseases symptoms

*Agrobacterium rubi* (Hilderbrand) Starr and Weiss 1943.

*Agrobacterium tumefasciens* (Smith and Townsend) Conn 1942 has a very wide range and pathogenicity depends on the Ti plasmid it contains; it causes galls of canes, raspberry, rosa spp, rubus spp, *Beta vulgaris*, *Kalanchoe daegremontiana*, *Helianthus annuus* and *Phaseolus vulgaris* through inoculation.

*Erwinia carotovora* subsp *carotovora* (Jones)

The bacterium is strongly pectolytic, rapid growing, and readily invades plant tissues if an entry is gained. It is therefore, able to attack a large number of different plants when conditions are right. The pathogen causes soft rot on sunflower. Large fleshly organs are particularly susceptible and once infected; they usually become softened to a pulp very quickly. This organism probably occurs in all areas where sunflower is grown.
**Pseudomonas marginalis pv. marginalis** *(Brown)* Stevens 1925, 30

The bacterium is positive for arginine dihydrolase and negative for tobacco hypersensitive reaction (HR). The pathogen has been reported to occur naturally in a wide range of host plants including *Helianthus annus*. It can cause leaf blight of *Syngonium podophyllum*. In Sunflower, usually, marginal necrosis of the leaf occurs. Necrotic areas may be soft initially and often become dry and papery later. Soft rots may also occur, particularly at maturity or in storage.

**Pseudomonas solanacearum** *(Smith)* 1941, 18

This is a non-fluorescent pseudomonad with a polar tuft of flagella. Cells are non-pigmented, but diffusible brown pigment often produced. Levan is not produced, gelatin hydrolysis weak, starch not hydrolysed, nitrate reduced by nearly all strains, many produces gas. There is no growth at 40°C; oxidase positive; arginine dehydrolase negative. This species is being distinguished from others by failure to grow at 40°C. Carbon sources used for growth include acetate, aconitate, asparagines, benzoate and L-histidine. Acid without gas is produced aerobically in medium with little peptone from fructose, glucose, glycerol and sucrose but not from dextrin.

The species is very heterogenous and has also been divided into three races on basis of pathogenicity (Buddenhagen, 1962). Diseases such as bacterial wilt and brown rot have been reported to be caused by the pathogen on most solaneceous plants. Infection is systematic in the vascular system, producing a wilt of parts or the whole plant. Other symptoms, which may occur with or without wilting, include browning of vascular tissue, bacterial exudates from cut vessels, stunting and chlorosis of plants.
**Pseudomonas syringae pv. aptata** (Brown and Jameson) 1978

This bacterium shows the characteristics of *P. syringae* and the following characteristics: levan is produced from sucrose, gelatin usually liquefied. L-lactate and D-mannitol are the carbon sources. The pathogen produces leaf spots on sunflower, and it has wide host range including woody species, vegetables, grasses and herbaceous ornamentals. The pathogen has spread in all cantaloupe growing areas worldwide.

**Pseudomonas syringae pv. helianthi** (Kawamura) 1978

In addition to the characteristics of the species, the bacterium produces levan from sucrose, liquefies gelatin slowly or not at all, fails to hydrolyse arbutin. Uses mannitol, sorbitol and D-gluconate as carbon source. *Helianthus annus, H. debilis* and *H. tuberosus* are the natural hosts. The pathogen produces leaf spots on the hosts.

**Pseudomonas syringae pv. tagetis** (Hellmers) 1978

The characteristics are those of the species *P. syringae* and in addition levan, production is variable (the type is negative), gelatin is slowly liquefied, arbutin is hydrolysed. D-alanine, D-gluconate, malonate are some of the carbon sources. It causes leaf spots of *Tagetes spp* and apical chlorosis of sunflower, while necrotic leaf spots and stunting is observed in Jerusalem artichoke plants.

### 2.2.4 Management of sunflower diseases

The use of disease free, good quality seed, fungicide treatment to kill surface seedborne spores, resistant varieties, and control of insect may help to reduce *Rhizopus* head rot. Proper disposal of infected crop residue and stubbles are important disease management measures for sunflower diseases mentioned above.
CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Field survey and seed collection

Surveys were conducted in Iringa, Dodoma and Morogoro regions in Tanzania to collect finger millet and sunflower seeds from farmers (farmer saved seeds). A total of six districts (Njombe and Iringa rural, Iringa region, Kilosa and Morogoro rural, Morogoro region and Kondoa and Dodoma rural in Dodoma region) were surveyed for collection of farmer- saved finger millet and sunflower seeds. In each district, 12 farmers were randomly selected taking into account the gender balance. In addition, seed samples were collected from the selected farmers. A total of 62 seed samples (36 finger millet seed samples each weighing 1kg; and 26 sunflower seed samples, each weighing 0.5kg) were collected. Each collected sample was packed in a paper bag and labeled (variety/cultivar name, source of seed, area collected, date harvested, and weight of seed harvested) and stored at 5 °C for further processing and assay for seed-borne pathogens.

3.2 Determination of seed quality

3.2.1 Preparation of working sample

The collected samples were poured onto a tray in the laboratory. Using a suitable spoon, small portions of samples were transferred, taken at random, following the ISTA halfing method (ISTA, 1996). Working sample sizes of 400 seeds were tested for finger millet and sunflower, while the working sample for count of other species were 6 g and 200 g for finger millet and sunflower seeds, respectively.
3.2.2 Inspection of dry seeds

Inspection of dry seeds was done to identify the presence of fruiting structures of fungi and the effects of fungi on the physical appearance of seeds. The fruiting structures of fungi that were inspected in/on seed included acervuli, pycnidia, perithecia, and sclerotia.

3.3 Determination of purity, germination and moisture content of seed samples

3.3.1 The purity analysis

The objective of this analysis was to determine: (a) the percentage composition by weight of the sample being tested and by inference the composition of the seed lot, and (b) the identity of the various species of seeds and inert particles constituting the sample.

3.3.2 Determination of seed germination

Four hundred seeds of fingermillet and sunflower were spaced uniformly apart on three moist substrates (Top of paper method) (Mathur et al., 2003) in a plastic container (Cello Domestoware) with a diameter and height of 15.3 cm and 9.3 cm, respectively. The containers were kept at 25-28°C for 10 and 6 days for the two crops, respectively. The ISTA (2005) procedures were used to evaluate the normal seedlings, abnormal seedlings and dead or hard ones.

3.3.3 Determination of moisture content

Ten gram working sample of fingermillet and sunflower seeds were evenly distributed over the surface of the 8.5 cm diameter petri dishes. The petri dishes were weighed before and after filling them with seed, and then placed in an oven
maintained at 103 ± 2°C and dried for 17 ± 1 hours for low constant temperature oven method, and at 130 – 133°C and dried for two hours for high constant temperature oven method (ISTA, 2005). The moisture content determination was carried out using two independently drawn working samples. The moisture content percentage was calculated using the formula, \( MC = \frac{(M_2 - M_3) \times 100}{M_2 - M_1} \); where: \( M_1, M_2 \) and \( M_3 \) are weight in grams of the container with its cover: weight in grams of the container plus cover and the content before drying: weight in grams of the container, cover and the content after drying, respectively.

3.4 Detection of fungi in finger millet and sunflower seed

3.4.1 Washing test

The washing test method (Mathur et al., 2003) was conducted to determine the fruiting bodies, mycelia and other fungal pathogenic structures. The suspensions obtained were examined under the compound microscope to detect the presence of seed-borne fungi whose inocula may be attached on the surface of seeds.

3.4.2 Blotter method

Seeds were plated on a well water soaked blotter and incubated in the refrigerator for seven days at 22 °C under 12 hours alternating cycles of light and darkness following procedures of Top of paper method (Mathur et al., 2003). After incubation, fungi which developed on each seed were examined under different magnifications using stereomicroscope and identified. The identification of the fungi was based on the growth habit on seeds “habit character”, and on the morphological characteristics of fruiting bodies, spores/conidia observed under a compound microscope.
3.5 Detection of bacterial pathogens in finger millet and sunflower seed

3.5.1 Extraction and isolation of bacteria from seed samples

Bacteria were isolated from seeds using the direct plating method onto a general medium and growing –on- test including seedling symptom tests. Then they were isolated on specific medium before purification and identification.

3.6 Identification of bacterial isolates

Purified bacterial isolates were identified according to colony morphology, pigment production on KB (King’s B medium), Gram-reaction based on potassium hydroxide solubility (Lelliot and Stead, 1987), pathogenicity (Shakya and Chung, 1983), oxidase reaction (Kovacs, 1956; Hildebrand and Schroth, 1968; Schaad, 1988), arginine dihydrolase, levan production, and pathogenicity on susceptible rice seedlings.

3.6.1 Gram reaction

The purified bacterial isolates were tested for Gram reaction using 3 percent potassium hydroxide (KOH) solubility test (Lelliot and Stead, 1987). On a glass slide, a loopful of bacterial cells from a 24 hour-well grown colony on KB was mixed with a drop of 3 percent KOH aqueous solution using a tooth – pick. The toothpick was then raised up at about 2 cm high from the glass slide. Bacterial isolates that produced the mucoid thread when raised from the KOH suspension were recorded as Gram negative, and those that did not produce the mucoid thread were recorded as Gram-positive bacteria.
3.6.2 Oxidase test

Oxidase test was conducted following the procedures of Kovac (1956) and as described by Hilderbrand and Schroth (1972), to determine the presence of Cytocrome C Oxidase enzyme among isolates as one of the distinguishing characters of bacteria species. A Whatman filter paper No. 1 was placed in a petri dish and 3 drops of freshly prepared one percent aqueous solution of tetramethyl-Phennylenediamine dihydrochloride was added at the center of the filter paper. Using a platinum loop wire, a loopful of bacteria grown on KB was streaked onto the moist filter paper. A change in colour of the reagent to a purple colour within 10 seconds of application of the culture was recorded as positive.

3.6.3 Arginine dihydrolase

This test permits certain pseudomonads to grow under anaerobic conditions. Usually, ammonia is evolved which brings about the change in pH, indicating a positive reaction (Lelliot and Stead, 1987). Fresh culture was inoculated by stabbing with Thornley`s medium, covered well and incubated for 3 days at 27°C.

3.6.4 Starch hydrolysis

This test was conducted to determine the ability of certain bacteria to hydrolyze starch and which is used as a taxonomic character in the differentiation of bacteria. Inoculated starch agar plates were streaked and incubated for at least 4 days. They were then flooded with Lugol`s Iodine. A positive reaction was revealed by the appearance of yellowish, clear zones around or under the bacterial growth.
3.6.5 Nitrate reduction test
Inoculated duplicate tubes containing a nitrate semi-solid medium re-melted and cooled to 40°C. The tubes were then mixed by rotation between palms and incubated at 27°C for 3-7 days plus a control test. Four drops of well prepared reagents were added in each test tube. A change of the colour of the reagents to blue-black was an indication of nitrate reduction (Fahy and Persley, 1983) and was recorded as a positive reaction.

3.6.6 Potato soft rot
This test was used following procedures described by Lelliot and Stead (1987). Round potato tubers were washed and surface disinfected by dipping in 70% alcohol followed by gentle flaming. The disinfected potato was aseptically cut into slices of 7-8 mm thick and placed in sterile petridish followed by 3-4 drops of distilled water. A groove was then made in slice and smeared with bacterial growth of 24 hours old agar culture.

3.6.7 Pathogenicity tests
The objective of the pathogenicity tests using hosts was to determine whether or not a suspected pathogen could cause disease symptoms in the host from which it was isolated. For many plant diseases, this test is the only method for confirming or negating the presumptive diagnosis. (Lelliot and Stead, 1978). Twenty four to forty eight –hour-old cultures grown on NA were used in pathogenicity tests. A loopful of bacterial growth was suspended in sterile distilled water at the concentration of $10^6$ cfu/ml. Using the spraying technique, young fingermillet and sunflower seedlings, 3 – 4 leaf stage, grown under the screen house conditions were selected and incubated
in polythene bags, with wires arranged to avoid the bags touching the plant, for 24 hours before inoculation. Using a sterile low pressure sprayer, the prepared bacterial suspensions were sprayed as a fine mist on foliage to run-off. The inoculated plants were immediately covered with polythene sheets to prevent drying and incubated for 48 hours. The containers were labeled with date of planting; isolate used and date of inoculation. The plants were then removed from polythene cover and observed periodically for diseases symptoms development for four weeks. Negative sunflower and finger millet control plants were sprayed with distilled water and incubated under the same conditions.

### 3.6.8 Tobacco hypersensitive reaction

Tobacco plants were used to further confirm the identity of the bacterial strains. The suspensions of bacteria previously grown on KB for 24 hours were used to prepare the inocula. The concentration of $10^8$ cfu/ml was prepared for each inoculum using sterile distilled water. The needles with inocula were injected in the mesophyl of the tobacco leaf to flood the intercellular spaces of the leaves. Each injected portion was labeled according to the isolate used. Tissue collapse and water soaking of the leaf after 24 hours of inoculation was recorded as positive for hypersensitive reaction, while non-collapse of the tobacco leaf tissues, dark brown colour, or when the leaf retain its colour was recorded as negative for hypersensitive reaction (Lelliot and Stead, 1987).

### 3.7 Data analysis

The data were analysed using CoStat computer package and mean separation tests were done using the Duncan`s Multiple Range Test, DMRT (P<0.05).
CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 General observation

Subsistence farmers in the study area grew all of their sunflower and fingermillet under low – input cropping systems. Most of the farmers surveyed grew 0.5 – 2 ha of sunflower and fingermillet. Conditions of their fields varied from well maintained to very poorly maintained to near abandoned, with a uniform distribution of these conditions across the surveyed regions (Iringa, Dodoma and Morogoro). The agronomic conditions of the sunflower and fingermillet fields among the surveyed farmers also varied. It was difficult to confirm, however, whether this reflected poor crop management or limitations in edaphic factors.

4.2 Germination test, purity and moisture content determination of finger millet

Twenty four fingermillet seed samples were tested for germination using the Top of the Paper method. The number of normal fingermillet seedlings varied significantly (P<0.001) between fingemillet seed samples tested (Table 1). Sample numbers 13, 14, 20 and 27 collected from Kisese Kondoa district, Manzese, Msusuri, Msalabani, Kilosa district and Kinonko, Morogoro Rural district, had the highest number of normal fingermillet seedlings (91%, 86%, 84% and 80%, respectively). While fingermillet seed sample numbers 11, 12 and 10 from ARI Ilonga Kilosa district, Malangali chini and Mambegu, Njombe district had the lowest number of normal fingermillet seedlings (31%, 33% and 37%, respectively). Fingermillet sample number 20 from Haubi, Kondoa district had the lowest (3%) dead seedlings. Fingermillet sample Nos 4 (Kihesa, Iringa rural), 14 (Manzese Msusuri, Kilosa), 15 (Lubungo, Morogoro rural) and 28 (Kinonko, Morogoro rural), abnormal seedlings
were 6%, 4%, 7% and 5%, respectively. While the highest number of abnormal fingermillet seedlings were from sample Nos 20 (28%), 17 (24%), 33 (23%) and 36 (23%) which were collected from Haubi Kondoa district; Dabaga Iringa district; Lukani, Iringa Rural district and Misuwa, Dodoma Rural district, respectively (Table 1).

The purity analysis of fingermillet seed samples were considerably varied. However, the six districts surveyed (Kilosa, Njombe, Kondoa, Morogoro Rural, Dodoma Rural, and Iringa Rural), had seed purity averages of 95%; 1.3 % inert matter and 1.1% seeds of other plant species. The admixture of fingermillet seeds and inert matter such as small stone pieces was observed in most of the fingermillet seed samples used in the current study. This may be attributed by poor harvesting; threshing and winnowing methods which may lead to poor seed quality and increase in seed borne infections.

Several factors may have lead to high number of abnormal and dead finger millet seedlings during germination. The main causes were damages by pests such as weevils and moulds during storage (ECARSAM, 2004). Infestation by seed borne pathogens is one of the reasons for increasing the number of abnormal finger millet seedlings during germination. Therefore, fingermillet seeds should be properly dried, cleaned and broken grains sorted out before storage. The sorghum and fingermillet seeds do not always store well compared to other cereals (FAO, 1970; Pushpammetal, 1985; TDRI, 1986).

In wheat, a direct relationship between the bunt infection and reduction of seed germination has been established by Bedi and Madhumeeta (1981). Saad et al.,
(1988), found that many of the seed borne fungi in *Vigna unguiculata* reduced seed germination an produced symptoms on infected seedlings.

Table 1: Germination percentage, purity and moisture content of fingermillet (*Eleusine coracana* Gaertn.) collected from Dodoma, Iringa and Morogoro regions in August, 2006.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Germination* (%)</th>
<th>Purity (%)</th>
<th>Moisture Content (%)</th>
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</table>

NS- Normal seedlings, AS- Abnormal seedlings, DS- Dead seedlings, PS- Pure Seeds, IM- Inert Matter, SOS - Seeds of Other Species, MC - Moisture Content, a- represents 400 seeds; Means followed by the same letters within a column are not significantly different P≤0.05 level based on Duncan’s Multiple Range Test (DMRT)
Plate 1: Fingermillet seed sample collected from Misuwa, Dodoma Rural mixed with maize

Plate 2: Clean fingermillet seed sample collected from Lubungo, Morogoro Rural
Other factors which may contribute to poor germination and low seed quality include delayed harvesting, threshing and storage technologies available for the farmers. Seed borne pathogens have been reported to cause deterioration of seeds under storage even if they were of high/good quality when stored (Baker, 1972). Seed borne pathogens are the organisms which when on or in seeds may or may not show symptoms which makes it difficult for the farmer to manage. In the current study, the moisture content of fingermillet seeds ranged from 11 % to 13.46 % (Table 1). Sixty seven percent of fingermillet samples had moisture content below the standard (13 %), while 33 % had moisture content of 13 to 13.46 % (Table1). This factor may influence the germination capacity of fingermillet seeds; however, high number of abnormal and dead seedlings increased the seed infection. This study suggests that fingermillet seeds should be produced in areas with low humidity, resulting in low infection levels in the field and farmers should make sure that seeds harvested are properly dried and have appropriate moisture levels.

4.4 Germination test, purity and moisture content determination of sunflower seeds

Twenty-six sunflower seed samples were tested for germination using Top of the Paper method. The results show significant variation (P=0.001) in germination. (Table 2). The highest record of normal sunflower seedlings were recorded from sample Nos 18 (96%), 7 (96%), 1 (95%) and 25 (94%) collected from Igoweko, Dodoma Rural; Makambako market, Njombe district; Mrijo, Kondoa and Vitisu, Dodoma Rural, respectively.
The lowest number of normal sunflower seedlings was recorded from sample No. 15 (86 %), collected from Fulwe, Morogoro Rural district. The number of abnormal sunflower seedlings and dead seedlings were also significantly different (P<0.001) between samples. The highest number of abnormal sunflower seedlings was recorded from sample No. 19 collected from Kimage Iringa, 9 % and No. 21 from Msalabani, Kilosa, 9% district; Morogoro region. Only 1% was recorded as the lowest abnormal sunflower seedlings for sample Nos. 3, 7, 13,14, 18 and 25 collected from Kiwele, Makambako market, Iringa; Mbula Njombe district and Maseyu, Morogoro Rural district; Morogoro region and Igowe and vitisu, Dodoma rural respectively.

The lowest number of dead sunflower seedlings were recorded on seeds from sample Nos 19 (2 %), 20 (2 %), 21 (1 %) and 1 (2 %) from Mrijo Kondoa, Kimage Iringa, Kutukutu and Msalabani, Kilosa district, respectively. While the highest number of dead seedlings of sunflower were from samples Nos. 5(10 %), 8 (10 %) and 14 (10 %) collected from Kutukutu, Kilosa district; Itolwa, Dodoma Rural and Mbula, Njombe district (Table 2). This is highly attributed by the reason that sunflower can be easily threshed-out from their heads without much beatings hence reduces the chances of seed infection in areas of low humidity especially when seeds have been harvested with good moisture levels and have dried enough to be stored. However, the case may be different in areas with high humidity which encourages head rot and moulding.
Table 2: Germination percentage, purity and moisture content determination of sunflower (*H. annuus* L.) seeds collected from Iringa, Dodoma and Morogoro regions in August, 2006.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Germination (%)</th>
<th>Purity (%)</th>
<th>Moisture Content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NS</td>
<td>AS</td>
<td>DS</td>
</tr>
<tr>
<td>1</td>
<td>95^a</td>
<td>3^b</td>
<td>2^b</td>
</tr>
<tr>
<td>Mean</td>
<td>91</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>

NS- Normal seedlings, AS- Abnormal seedlings, DS- Dead seedlings, PS- Pure seeds, IM- Inert matter, SOS- Seeds of Other Species, MC- Moisture Content, a- represents 400 seeds; Means followed by the same letters within a column are not significantly different at P ≤ 0.05 level based on Duncan’s Multiple Range Test (DMRT)
The purity analysis of sunflower seed samples indicated that the minimum standard (98 %) of sunflower seeds was met by only 56 % of the seed samples. While the highest purity was recorded in sample Nos 11 (99.9 %), 2 (99.55 %), 3 (99.95 %), 5 (99.70 %), 17 (99.80 %), 19 (99.55 %), 20 (99.75 %), 21 (99.76 %) and 22 (99.38 %) collected from Mkoka, Dodoma Rural district, Ismani Market Iringa, Kiwele Iringa Rural, Kutukutu Kilosa, Mkwage, Kimage Iringa Rural, Msalabani Kilosa and Soya Dodoma Rural district, respectively. Sample No. 8 (91.44%); collected from Itolwa, Dodoma Rural recorded the lowest purity of sunflower seed. Seeds of other crops such as maize, fingermillet, beans were also observed in sample Nos. 14 (0.19 %), 24 (0.12 %) and 4 (0.36 %) (Table 2). Only 15 % of the sunflower seed samples had the recommended moisture content (13 %), while 85 % of the seed samples had moisture content levels below the minimum standard (Table 2).

Sample No. 14 collected from Mbula, Njombe had the highest moisture content of 14.4 %. These results suggest that, most of the farmers in these locations had poor harvesting technologies including threshing and winnowing. Post harvest measures such as winnowing and sun drying are poorly practiced. Therefore, despite of the good moisture content of seeds in most locations, the above factors may increase the incidence of seed borne infections.
Plate 3: Fingermillet seed collected from Kihesa, Iringa and Manzese, Kilosa infected with *Pyricularia grisea* (A) and *Bipolaris nodulosa* (B)

Error: Reference source not found

Plate 4: Sunflower seed collected from Mikese, Morogoro Rural and Mkoka, Dodoma Rural infected with *Altenaria helianthi* (A), saprophytes (B) and *Altenaria altentia* (C)

4.5 Seed-borne fungal pathogens detected in fingermillet seeds

The Blotter method was used to detect fungal pathogens in fingermillet seed samples (Plate 3). Three fungal pathogens, *Pyricularia grisea*, *Bipolaris setariae* and *Bipolaris nodulosa* were detected in fingermillet seed samples (Table 3).

The incidence of fingermillet seed infection by *Bipolaris nodulosa* ranged from 10 – 50 %, while that of *B. setariae* ranged from 0.0 to 50 % and that of *Pyricularia grisea* had a range of 4.5 to 50 % (Table 3). The result also showed that fingermillet sample Nos. 4, 14, 18, 21, 23 and 27, collected from Kihesa, Iringa, Manzese Msusuri Kilosa, Chakwa Dodoma Rural, Msalabani Kilosa, Kinonko Morogoro Rural and Mrijo
Kondoa, respectively, had the highest incidences of seed borne fungi, (up to 50%) compared to the rest of samples. (Table 3).
Table 3: Incidence of fungal pathogens detected in fingermillet seed samples collected from Dodoma, Iringa and Morogoro in August, 2006.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Location</th>
<th>Incidence of seed infection (%)</th>
<th>Other microbes detected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Bn (%)</td>
<td>Bs (%)</td>
</tr>
<tr>
<td>4</td>
<td>Kihesa, Iringa</td>
<td>35⁺</td>
<td>50⁺</td>
</tr>
<tr>
<td>8</td>
<td>Chenene, Dodoma</td>
<td>30</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>Mambegu, Njombe</td>
<td>10</td>
<td>25</td>
</tr>
<tr>
<td>11</td>
<td>Ilonga, kilosa</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>12</td>
<td>Malangali, Njombe</td>
<td>10</td>
<td>30</td>
</tr>
<tr>
<td>13</td>
<td>Kisese, Kondoa</td>
<td>40</td>
<td>0.0</td>
</tr>
<tr>
<td>14</td>
<td>Manzese, Kilosa</td>
<td>50⁺</td>
<td>0.0</td>
</tr>
<tr>
<td>15</td>
<td>Lubungo, Morogoro</td>
<td>50⁺</td>
<td>-</td>
</tr>
<tr>
<td>16</td>
<td>Dabaga market, Iringa</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>17</td>
<td>Chakwa, Dodoma Rural</td>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>18</td>
<td>Haubi, Kondoa</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>20</td>
<td>Msalabani, Kilosa</td>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>21</td>
<td>Kinonko, Morogoro</td>
<td>50</td>
<td>-</td>
</tr>
<tr>
<td>22</td>
<td>Mungoni, Kondoa</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>23</td>
<td>Mikese market, Morogoro</td>
<td>50⁺</td>
<td>10</td>
</tr>
<tr>
<td>24</td>
<td>Mungoni, Kondoa</td>
<td>15</td>
<td>-</td>
</tr>
<tr>
<td>25</td>
<td>Mikese market, Morogoro</td>
<td>25</td>
<td>10</td>
</tr>
<tr>
<td>26</td>
<td>Igunda, Iringa Rural</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>27</td>
<td>Mrijo, Kondoa</td>
<td>50⁺</td>
<td>-</td>
</tr>
<tr>
<td>28</td>
<td>Kinonko, Morogoro</td>
<td>50</td>
<td>-</td>
</tr>
<tr>
<td>31</td>
<td>Luduga, Njombe</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>33</td>
<td>Lukani, Iringa</td>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>34</td>
<td>Busi, Kondoa</td>
<td>50</td>
<td>-</td>
</tr>
<tr>
<td>35</td>
<td>Saja, Njombe</td>
<td>50</td>
<td>-</td>
</tr>
<tr>
<td>36</td>
<td>Misuwa, Dodoma Rural</td>
<td>20</td>
<td>-</td>
</tr>
</tbody>
</table>

Bn - *Bipolaris nodulosa*, Bs - *Bipolaris setariae*, Pg - *Pyricularia grisea*

⁺highest incidence of fungal infection  ·  ++  Lowest level of fungi infection

Four hundred seeds were used for the experiment.

4.6 Seed borne fungal pathogen detected in sunflower seeds

The Deep Freezing Blotter method was used to detect the presence of fungal pathogens in sunflower seeds. Results of seed-borne fungi detected in sunflower seeds used in this study are presented in Table 4. Only two fungal pathogens (*Altenaria altenata* and *Verticillium albo-atrum*) were identified on most of the
samples tested. *Altenaria altenata* was detected on sample Nos. 10, 14, 20 and 24, collected from Lupembe and Mbula in Njombe district; Kutukutu, Kilosa and Kitanzini market, Iringa Rural, respectively. Sample Nos. 14 and 24 had the highest incidence of seed infection (50 %), while sample Nos. 10 and 20 had only 15 % seed infection incidence. The pathogen *Verticillium albo-atrum*, was detected on sample number 4 only with up to 50 % seed infection (Table 4). There was no pathogen isolated from the rest (95 %) of the sunflower seed samples tested. All of the twenty six sunflower seed samples tested were contaminated by saprophytic fungi, mostly, *Rhizopus spp*, *Aspergillus niger*, *Aspergillus flavus*, *Fusarium spp*, *Actinomycetes*, *Penicillium spp* (Table 4).

Acimov (1979) described *Altenaria helianthi* as a very harmful and wide spread causative agent of leaf spot disease of sunflower and that plants may be attacked by *A. helianthi* at any growth stage leading to very severe withering, stem death, and seed head rot. The fungus has a wide host range including castor. Thompson *et al.*, (1980) suggested that, a wilt caused by *Verticillium albo-atrum* has been reported to attack many tropical plants, and can be serious on susceptible crops which should therefore, not be grown in succession. The pathogen can be seed-borne; and seeds from disease free stocks will materially reduce its occurrence. Futon *et al.*, (1965), indicated that, *Altenaria altenata* can lead to production of tentoxin in cotton seed which causes irreversible chlorosis in germinating seedlings of susceptible dicotyledonous spp. In sunflower, the pathogen can produce several toxic metabolites such as altenariol monomethyl ether and tenuzoic acid. Also *A. altenata* can influence the production of aflatoxin by *Aspergillus parasiticus*. 
Table 4: Incidence of seed borne fungal pathogens detected in sunflower seed samples collected from Dodoma, Iringa and Morogoro regions in August, 2006.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Location</th>
<th>Incidence of fungi identified in % in 400 seed/sample tested</th>
<th>Other microbes detected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>Alternaria alternata</em></td>
<td><em>Verticillium albo-atrum</em></td>
</tr>
<tr>
<td>1</td>
<td>Mrjio, Kondoa</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>2</td>
<td>Ismani Market, Iringa</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>3</td>
<td>Kiwele, Iringa Rural</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>4</td>
<td>Saja, Njombe</td>
<td>0.00</td>
<td>50.00</td>
</tr>
<tr>
<td>5</td>
<td>Kutukutu, Kilosa</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>6</td>
<td>Lubunugo, Morogoro Rural</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>7</td>
<td>Makambako Market, Njombe</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>8</td>
<td>Itolwa, Dodoma Rural</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>9</td>
<td>Mikese Market, Morogoro Rural</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>10</td>
<td>Lupembe, Njombe</td>
<td>15.00</td>
<td>0.00</td>
</tr>
<tr>
<td>11</td>
<td>Mkoka, Njombe</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>12</td>
<td>Luduga, Njombe</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>13</td>
<td>Maseyu, Morogoro Rural</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>14</td>
<td>Mbula, Njombe</td>
<td>50.00</td>
<td>0.00</td>
</tr>
<tr>
<td>15</td>
<td>Fulwe, Morogoro Rural</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>16</td>
<td>MATI Ilonga, Kilosa</td>
<td>20.00</td>
<td>0.00</td>
</tr>
<tr>
<td>17</td>
<td>Mk wage, Dodoma Rural</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>18</td>
<td>Igoweke, Dodoma Rural</td>
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<td>0.00</td>
</tr>
<tr>
<td>19</td>
<td>Kimage, Iringa Rural</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>20</td>
<td>Kutukutu, Kilosa</td>
<td>10.00</td>
<td>0.00</td>
</tr>
<tr>
<td>21</td>
<td>Msalabani, Kilosa</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>22</td>
<td>Soya, Dodoma Rural</td>
<td>2.00</td>
<td>0.00</td>
</tr>
<tr>
<td>23</td>
<td>Gwata Ujembe, Morogoro Rural</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>24</td>
<td>Kitanzini Market, Iringa</td>
<td>50.00</td>
<td>0.00</td>
</tr>
<tr>
<td>25</td>
<td>Vitsu, Dodoma Rural</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>26</td>
<td>Jangalo, Kondoa</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

* High infection, 400 seeds were used.
4.7 Identification of bacterial isolates from fingermillet and sunflower seed samples

The direct plating method was used to extract pathogens from fingermillet and sunflower seed samples. The results indicate that only four pathogens, *Pseudomonas eleusines*, *Xanthomonas campestris pv coracanae*, *Pseudomonas syringae pv syringae* and *Pseudomonas andropogonis* were isolated from the fingermillet seed samples (Table 5).

### Table 5: Incidence of seed borne bacterial pathogens in fingermillet seed samples collected from Iringa, Dodoma and Morogoro regions in August, 2006.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Crop/variety</th>
<th>Location</th>
<th>Incidence of bacteria detected (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>Pe</em></td>
</tr>
<tr>
<td>3</td>
<td><em>Eleusine coracana</em></td>
<td>Ninga, Njombe</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td><em>Eleusine coracana</em></td>
<td>Magomeni, Kilosa</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td><em>Eleusine coracana</em></td>
<td>Mambegu, Njombe</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td><em>Eleusine coracana</em></td>
<td>Ilonga, Kilosa</td>
<td>28.25</td>
</tr>
<tr>
<td>16</td>
<td><em>Eleusine coracana</em></td>
<td>Mengu, Dodoma</td>
<td>-</td>
</tr>
<tr>
<td>21</td>
<td><em>Eleusine coracana</em></td>
<td>Msalabani, Kilosa</td>
<td>12.75</td>
</tr>
<tr>
<td>23</td>
<td><em>Eleusine coracana</em></td>
<td>Kinonko, Morogoro Rural</td>
<td>-</td>
</tr>
<tr>
<td>25</td>
<td><em>Eleusine coracana</em></td>
<td>Mikese, Morogoro</td>
<td>-</td>
</tr>
<tr>
<td>30</td>
<td><em>Eleusine coracana</em></td>
<td>Zanka, Dodoma Rural</td>
<td>-</td>
</tr>
<tr>
<td>32</td>
<td><em>Eleusine coracana</em></td>
<td>Ilonga, Kilosa</td>
<td>-</td>
</tr>
</tbody>
</table>

*Xcco*=*Xanthomonas campestris pv coracanae*, *Pa*=*Pseudomonas andropogonis*, (-) none detected: *Pss*=*Pseudomonas syringae pv syringae*; *Pe*=*Pseudomonas eleusines*; xxx -first occurrence in Tanzania
Plate 5: Isolates from seed sample Nos. 10 and 29 collected from Mambegu, Njombe and Kinonko, Morogoro showing positive and negative Arginine Hydrolase test.

Plate 6: Isolate from seed sample No. 23 collected from Mikese Market, Morogoro Rural showing positive starch hydrolysis (A).
Pseudomonas eleusines was detected in sample Nos. 11 (28.25 %) and 21 (12.75 %) collected from Ilonga and Msalabani, Kilosa district. Xanthomonas campestris pv coracanae was detected in samples Nos. 3 (Ningga, Njombe) and 16 (Mengu, Dodoma Rural) with incidence of 36.75 % and 54.5 %, respectively. Pseudomonas andropogonis was detected on samples Nos. 23 (Kinonko, Morogoro Rural), 25 (Mikese Market, Morogoro Rural), 30 (Zanka, Dodoma Rural) and 32 (Ilonga, Kilosa), with infection percentages of 62.25 %; 72.25 %; 71.5 % and 46.75 %, respectively (Table 5).

Plate 7: Colonies of Pseudomonas andropogonis isolated from fingermillet seed sample No. 21 collected at Kinonko, Morogoro Rural on King’s B medium

Pseudomonas syringae pv syringae was detected in sample Nos. 7 (Magomeni, Kilosa) and 10 (Mambegu, Njombe) with infection percentage of 100 % and 50.75 %, respectively (Table 5). The pathogens Erwinia caratovora subsp caratovora,
P. marginalis pv marginalis, P. syringae pv tagetis, P. syringae pv helianthi and P. syringae pv aptata were detected from sunflower seed sample Nos. 4, 5, 16, and 19 collected from Saja, Njombe, Kutukutu, Kilosa, Kimage, Iringa Rural and MATI Ilonga-Kilosa, with 36 %, 47 %, 23 %, 19 % and 23 % infection, respectively (Table 6).

Pseudomonas andropogonis has been reported to have a wide host range mainly in two hosts families, Graminae and Leguminosae; while in the other families, the pathogen has been isolated from naturally infected Bouganinvillea spp (Bradbury, 1986). Leukel et al., (1951); Leukel and Martin (1953) and Tarr (1961) suggested that disposal of plant wastes from previous crop and seed dressing, can help to reduce bacterial stripe, bacterial streak, and bacterial spot caused by the pathogens Pseudomonas andropogonis, Xanthomonas halicola and Pseudomonas syringae, respectively.

Table 6: Incidence of seed borne bacterial pathogens in sunflower seed sample collected from Dodoma, Iringa and Morogoro regions in August, 2006.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Location</th>
<th>District</th>
<th>Ecc</th>
<th>Pmm</th>
<th>Psa</th>
<th>Psh</th>
<th>Pst</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>Saja</td>
<td>Njombe</td>
<td>36</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Kutukutu</td>
<td>Kilosa</td>
<td>-</td>
<td>-</td>
<td>17</td>
<td>23</td>
<td>19</td>
</tr>
<tr>
<td>16</td>
<td>MATI Ilonga</td>
<td>Kilosa</td>
<td>-</td>
<td>47xxx</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Ecc = Erwinia carotovora, Psh = Pseudomonas syringae pv helianthi, Ps t = Pseudomonas syringae pv tagetis, Pmm = Pseudomonas marginalis pv marginalis
xxx = Highly infected
Four hundred seeds were used for detection.
CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

This study provides the first quantitative report on the incidence of seed borne pathogens in finger millet and sunflower seeds in Morogoro, Dodoma and Iringa regions. Previous studies that listed seed borne pathogens as constraints to finger millet and sunflower production in these regions did not quantify the extent and specific seed borne pathogens of the two crops. Such information is important in determining the epidemiology of these pathogens in the seed production system.

Three finger millet seed borne fungi; *Pyricularia grisea* – blast of finger millet, *Bipolaris nodulosa* and *B. setariae* causing leaf blights; two sunflower seed borne fungi, *Altenaria altenata* (Leaf spot) and *Verticillium albo – atrum* causing verticillium wilt were detected in the current study. Four finger millet seed borne bacteria, *P. eleusines* (bacterial wilt), *X. campestris pv coracanae* (bacterial blight), *P. syringae pv syringae* and *P. andropogonis* (bacterial stripe); Five sunflower seed borne bacteria; *E. caratovora subsp caratovora* (soft rot), *P. marginalis pv marginalis* (leaf blight), *P.syringae pv helianthi* (leaf spot), *P.syringae pv tagetis* and *P. aptata* (leaf spot), were also identified in this study. The pathogen *P. andropogonis* (causing bacterial stripe), was detected on finger millet as the first occurrence in Tanzania on samples collected from Ilonga, Kilosa; Zanka, Dodoma Rural district; Kinonko and Mikese market in Morogoro Rural district. Some of the reasons which lead to the occurrence of these diseases include the use of non-improved seeds by farmers. Farmers prefer to use their own saved seeds from the previous harvest and could be
infected with seed borne pathogens which can not be easily detected since they have little or no knowledge on the detection techniques.

Other reasons include infection in the field, poor threshing methods, poor post harvest technologies such as winnowing and storage methods which encourage the susceptibility of the seeds to seed borne pathogens as observed during sample collection in the fields. Also it was observed that factors such as high humidity and high moisture level of seeds during harvesting were the main reasons for occurrence of saprophytes such as Aspergillus niger, penicillium spp, Rhizopus spp and Fusarium spp in both fingermillet and sunflower seeds.

The result of this study has also established very important information on the occurrence of fingermillet blast (P. grisea) and bacterial stripe (P. andropogonis) across the surveyed regions. The influence of agro-eco systems on this study, however, had little effect on pattern of distribution with respect to incidence of seed infections, though studies to be conducted would have to consider the fact those pathogens of fingermillet and sunflower to occur as a complex, whose composition, incidences and severity depends on production environments and agronomic practices.
5.2 Recommendations

Studies relating disease incidence and severity to yield losses would therefore, need to be replicated across several agro-ecological zones or where these crops are grown in order to determine variations in agro – ecosystems, since this study was very confined in only three regions (Morogoro, Iringa and Dodoma), with only two districts in each. Furthermore, such studies will need to include local landraces/varieties such as those collected from farmers as farmers saved seeds in order to reflect losses encountered by farmers. In the mean time, finger millet and sunflower varieties intended for release must be tested for resistance to the seed borne pathogens detected in this study. Furthermore, this study suggest that fingermillet and sunflower seeds should be produced in areas with low humidity to lower the infection in the field and seeds should be dried enough to have appropriate moisture level before storage.
REFERENCES


## APPENDICES

### Appendix 1: Location and dates for collection of fingermillet farmer saved seed for the study

<table>
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<tr>
<th>Sample No.</th>
<th>Host/variety</th>
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<th>Sample size collected (kg)</th>
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### Appendix 2: Location and dates for collection of sunflower farmer saved seed for the study

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