Identification of the Causal Agent of Sorghum Leaf Blight and Evaluation of its Current Management Practices in Pandamatenga Farming Area

Masters of Science (MSc) in Crop Science (Crop Protection)

by

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A dissertation submitted to the Department of Crop Science and Production in Partial fulfilment of the requirements for the Degree of Masters of Science (MSc) in Crop Science (Crop Protection)

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STATEMENT OF ORIGINALITY

The work contained in this dissertation was compiled by the author at the University of Botswana, Botswana College of Agriculture between January 2013 and December 2014. It is original except where references are made and it will not be submitted for the award of any other degree or diploma of any other university

Mogomotsi Motsa ..... 25/08/2015 .....

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ABSTRACT

Turcicum Leaf Blight (TLB) of sorghum incited by *Exserohilum turcicum* is a major threat to sorghum production globally. This pathogen has been reported to attack both maize and sorghum resulting in yield losses as high as 70% on susceptible cultivars.

The leaf blight epidemic in Pandamatenga area is favoured by the abundant inoculum on infested surface residues and soils that tend to water log due to their clay nature. Farmers seem to be applying chemical control measures when the causal agent is not fully known which may lead to waste of money and pollution of the environment.

A study was therefore conducted to identify the pathogen causing the sorghum leaf blight (SLB) in Pandamatenga farms. The objectives of the study were: to identify the pathogen using morphological features, assess the incidence of the disease in the area during the 2012/2013 cropping season, assess the survival of the pathogen over a five week period on infested crop residues and in the soil, to assess in-vitro the effectiveness of the fungicides currently being used by farmers in Pandamatenga and assess the response of thirteen different sorghum varieties to the pathogen infection in the green house.

The pathogen causing the leaf blight in Pandamatenga farming area was identified as *Exserohilum turcicum*. The incidence of the disease in the farms ranged from 2% to 100%, and 100% of farms assessed had the disease in 2012/2013 season. Fungicide in vitro inhibition assays indicated that *E. turcicum* was sensitive to Propicon 250 EC (propiconazole 250g/l) and Artea (propiconazole 250g/l and cyproconazole 80g/l), but was not affected by Impact (Flutriafol 125g/l). Burial of stubble was found to reduce survival of *E. turcicum*, compared to leaving stubble on the surface.

Sorghum varieties such as Segaolane, Sephala, MR-Taurus and PANNAR 8816 exhibited higher levels of tolerance than NUS 421, NUS 510, NS5655, Tiger and PANNAR 8909 to *E. turcicum* infection under greenhouse conditions.

The results for the identification of *E. turcicum* will add to the disease list of sorghum in Botswana and will be useful to farmers when they plan their sorghum crops especially in Pandamatenga. The results on the reaction of varieties to *E. turcicum* will be useful to the Department of Agriculture Research (DAR) on evaluation of varietal performance data that might enhance their plant breeding research and the Plant Protection Division (PPD) when they do their disease surveys for compliance data in their endeavour to establish a biosecurity arm of the Ministry of Agriculture. Further research is thus needed to determine how many races of *E. turcicum* are found in the whole country and also to determine the host range so that an Integrated Disease Management (IDM) can be drawn especially to cater for subsistence farmers.

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Chapter 1.0 INTRODUCTION

1.0.1 Sorghum: systematics and socio economic importance

Sorghum belongs to the family Gramineae, tribe Andropogoneae, genus *Sorghum* and species *bicolor*. It originates in Africa and it is distributed throughout the inter-tropical zone and its cultivation now extends well into the temperate regions (Chanterean and Nicou, 1994).Sorghum [*Sorghum bicolor* (L.) Moench] is an indigenous crop to Africa, and though commercial needs and uses may change over time, sorghum will remain a basic staple food for many rural communities in Africa (du Plessis, 2008). The cultivated sorghum is a C4 plant adapted to hot, semi-arid tropical and dry temperate areas of the world (ICRISAT, 2004). It grows to a height ranging from 50 cm to 6 m and it is cultivated for both grain and fodder. The plant has fibrous root system that may penetrate 1.5 to 2.4m into the soil that makes sorghum one of the hardiest cereals and it is for this reason that it is grown in regions where most other crops fail to grow. Sorghum is propagated through its sexual seed.

Sorghum (*Sorghum bicolor* (L.) Monech) has been classified into five basic species, *bicolor*, *caudatum, durra, guinea* and *kafir* (Doggert, 1988). The origin and early domestication of sorghum is hypothesized to have taken place around 5000-8000 years ago (ICRISAT, 2004) in north eastern Africa or at the Egyptian- Sudanese border, with the largest diversity of cultivated and wild sorghum also found in this part of Africa.

Sorghum production has over the years evolved from the local landraces that are tall and late maturing to hybrids characterized by early maturity and production of higher yields (ICRISAT, 2004). The improvement has seen the release of such sorghum varieties as Tegemeo (1986), Pato (1995) and Macia (1999). Of these varieties Macia is largely produced in Botswana as Phofu. Sorghum is one of the major global crops making it fifth after *Triticum aestivum* (wheat), *Oryza sativa* (rice), *Zea mays* (maize) and *Hordeum vulgare* L (barley)

respectively (de Milliano, 1992; ICRISAT, 2004). It is a staple food for millions of poorest and most food insecure people in the semi-arid tropics (SAT) of Africa, Asia and Latin America (ICRISAT, 2004). In all these agro ecological zones sorghum is a dual-purpose crop; both grain and stover are highly valued outputs. In large parts of the developing world stover represents up to 50 per cent of the total value of the crop, especially in drought years. de Milliano (1992) reported that in Africa, where annual cereal production amounts to some 8.9 million tonnes, or 5% of the world total, the order of importance of the major cereals is markedly different. Sorghum is second only after maize and accounts for 14% of the total production. Annual production of sorghum is estimated at about 60 million tonnes, with Africa contributing 21 million tonnes. In 2003, for example, about 46 million ha of sorghum was harvested, of which 26 million were in Africa (FAO, 2004). Average productivity estimates showed that the mean yield in Africa is about 800 kg/ha, 1200 kg/ha in Asia, 4000 kg/ha in America and 5000 kg/ha in Europe.

In Botswana sorghum is the major cereal produced and as such considered a staple food, and it is widely grown across the country. Sixty thousands tons of sorghum is consumed annually and about half of which is produced locally and the rest is imported (Ohiokpehai and Kebakile, 1996). Pandamatenga is the highest sorghum producing area and it's currently considered the bread basket of Botswana. Among the varieties of sorghum that have been cultivated in Botswana are Phofu, Mahube, BSH 1, Mmabaitse (BOT 79) (ICRISAT, 2004: Rohrbach and Makhwaje 1999) and recently farmers are using MR-Buster and Pannar sorghum hybrids. Sorghum still remains the most consumed cereal in peri-urban areas accounting for 74% of the total cereal consumption in these areas, as such production of sorghum in Pandamatenga has grown over the years due to its marketability as seen below.

Table 1 - Cereals produced in Pandamatenga commercial farms from 2005-2011

Year	Sorghum		Maize		Millet	
	Hectares	Tons	Hectares	Tons	Hectares	Tons
2005	8275	14695.6	470	363	592.7	229
2006	8773.75	15352.6	732	363	647.79	229
2007	12870.3	25397	535	543.7	440	435.25
2008	11359	29020	60	72.48	170	150
2009	15511	39331	165	172.5	454.3	568.95
2010	14242	26562	10	3	540	310
2011	6594	15732	42	32.5	1018	1029

Ministry of Agriculture (MoA, Botswana), Food Security Report 2011

Sorghum is a staple food to about 300 million people who live in the dry tropics. It not only provides grain for human consumption but the leaves and stems are also used as forage for livestock, as building materials and fuel for cooking. In each country and most regions, there is a considerable level of production that never reaches commercial markets but is destined for local consumption (Chanterean and Nicou, 1994).

Sorghum grain is an energy-providing food due to its high starch content. The amino acid composition of sorghum proteins, as in all cereals, shows a lysine deficiency. Unlike maize however, sorghum contains sufficient levels of tryptophan and sulphur containing amino acids. The protein content of sorghum is higher than that of maize but less than that of wheat. The lipids of the seed germ are very rich in linoleic and linolenic poly unsaturated fatty acids, which are essential in human nutrition and are reported to be beneficial to the arteries. The vitamin content is relatively high and is mainly concentrated in the outer layer of the grain.

There are many food uses for sorghum and although they differ from country to country they can nevertheless be grouped into three main categories; preparation obtained from the boiled or ground grain (China, India, Africa), preparation requiring decortication or milling (porridge, clear gruel, pancakes and flat bread, couscous and doughnuts(Africa), drinks both alcoholic and non-alcoholic (Chanterean and Nicou, 1994).

1.1.0 Production constraints

The yield and quality of sorghum produced in the African region is affected by a wide array of biotic (pests and diseases) and abiotic stresses (drought and problematic soils) (ICRISAT, 2004). Some of the biotic stresses are shoot fly (Eastern Africa), stem borer (Africa), midge (Eastern Africa) and head bug (Western and Central Africa (WCA) among other insect pests; grain mould (all regions in Africa), anthracnose (WCA) among diseases and *Striga* spp (all regions in Africa) as parasitic weed. Abiotic stresses include drought (all regions) and problematic soils especially salinity.

During the author's interaction with farmers as a plant protection extension officer for Chobe District from 2006 until 2011, farmers in the Pandamatenga area were wondering about the causal agent of the leaf blight. Sprays with Dithane, Mancozeb, and Maneb have been used for a long time by farmers in Pandamatenga and recently fungicides such as Impact, Propicon and Artea are used to control the leaf blight with a vague understanding of the exact causal agent of the leaf blight. This results in either unnecessary costs or destruction of beneficial micro-organisms and even the risk of resistance to those pathogens that could respond to the treatments as well. The problem was also reported during consultative workshops on biosecurity issues in the Chobe district with the Department of Agriculture and Food Western Australia (DAFWA) in 2010 year. Farmers thought although the drainage system is a solution to waterlogging (drainage), there is always going to be chances of crop losses from the leaf blight that has been prevailing in that area.

1.1.1 Abiotic and socio-economic factors

Erratic rainfall is a major problem for Botswana, farmers since their agriculture is typically rain-fed. Farmers hold the view that the amount of rainfall has been decreasing over the years and is insufficient (Kudadjie *et al.*, 2004). It appears that the problem stems from lack of appropriate sorghum varieties that fit the current rainfall regime. Poor soils are also a general concern to farmers. Sorghum production is normally a common practice in marginal areas that are prone to infertility and water stress (Kudadjie *et al.*, 2004). The majority of smallholder farmers, especially in the semi-arid tropical regions of Africa, do not produce enough to meet family requirements (FAO, 1996). Furthermore sorghum is a semi-subsistence enterprise that offers smaller returns than other investments such as livestock. As a result, less attention is paid to improve its production. These abiotic factors hamper sorghum production resulting in low yields and income generation for the rural poor.

1.1.2 Biotic factors

Striga spp is a major constraint of sorghum production in Botswana especially in the sandy loamy soils which are low in nitrogen There are at least two species of Striga known to affect sorghum production in the country namely: *Striga hermonthica* and *Striga asiatica* (Mbikiwa, 1991). Some *Striga*-resistant sorghum varieties have been developed, but these generally offer lower yields than traditional cultivars and improved (but *Striga*-susceptible) varieties (FAO, 2007). The effect of *Striga* spp has been found to decrease when crops are grown in conjunction with legumes (Carsky *et al.*, 1994). The most important arthropod pests of sorghum in Botswana include sorghum shoot fly (*Atherigona varia soccata)*, sorghum midge *(Contarinia sorghicola)*, sorghum aphid (*Aphis sorghi*), African bollworm (*Heliothis emigera*), corn cricket (*Acanthoplus discoidalis*) and sorghum stem borer (*Busseola fusca)* (Sharma, 1993; Mchive, 1991). Sorghum shoot fly has also been reported to cause substantial losses in late and off-season sorghum in Uganda (Davies and Reddy, 1981) and Stem borers

are also endemic in most parts of Uganda (Gitau *et al.*, 2002). The most important species of sorghum pests in Africa include *Chilo partellus*, *Sesamia calamitis* and *Busseola sorghida*. *Chilo partellus* is mainly found in the semi-arid areas of East Africa while *Sesamia calamitis* and *Busseola sorghida* are distributed throughout sorghum growing areas of Africa (Kfir, 1997). Birds are perhaps one of the most important pests of sorghum worldwide. They are capable of inflicting heavy losses and causing economic damage. In Botswana the most notorious species is *Quelea quelea lathamii* (Bashir, 1991). In Pandamatenga sorghum leaf blight is the major problem that the farmers have not been able to manage. The problem is also exacerbated by lack of a pathology research officer in the area at the Department of Agriculture Research (DAR), who could identify the problem for farmers to have an idea of what diseases they had. The blight has always been referred to as northern leaf blight by the farming community. It is very important therefore that the pathogens causing the sorghum leaf blight (SLB) be identified.

1.2.0 Justification of the study

Sorghum is a staple food in Botswana, and production is very well distributed throughout all the geographical farming areas. It is a crop of mostly subsistence importance that is well adapted to both tropical and sub-tropical conditions. Sorghum in Botswana is produced under both commercial and subsistence systems, of which Pandamatenga is the main sorghum producing area on commercial basis with yields ranging from an average low of 1.75ton/ha in 2006 and an average high of 2.55ton/ha in 2008 (MoA, 2011). The low yields have been attributed to poor soil drainage and high incidence of leaf blight disease as a result of reduced tillage which results in inoculum build up. Assessment of SLB disease incidence and identification of the causal agent(s) in the Pandamatenga is justified in order to come up with timely and proper management of the disease. The SLB has caused devastating losses to farmers' crops in the Pandamatenga area. In 2008/09 cropping season alone an estimated 40-

60% loss was attributed to SLB (Pandamatenga production report, 2009).In severe cases all the leaves dry before booting and the plants produce small seeds and/or very few seeds per panicle. During severe outbreaks of the SLB farmers would come to the plant protection office to seek assessment for compensation classifying the problem as a disaster.

Factors like multiple diseases of sorghum on the same leaf, host pigment reactions, and other complex situations are said to obscure otherwise typical symptoms (Fox, 1993). Host pigmentation can dramatically affect the appearance of disease symptoms and hence of disease assessment. The aim of this work therefore is to study the SLB in Pandamatenga farming area and determine the pathogen(s) causing the complex. The reaction of varieties that are grown in the Pandamatenga area to the pathogens will also be assessed for any form of resistance. The whole process is aimed at making sure that the farmers in that area understand exactly what they are dealing with and how they should manage the problem through an Integrated Disease Management (IDM) approach.

Studies of sorghum diseases in Botswana have been given very little attention so far. Therefore this study will contribute to the literature on the disease in Botswana. It is very vital to document all the diseases both acute and chronic that are of local importance, for phytosanitary purposes. Export of surplus grain sorghum from Botswana is anticipated with government initiatives such as the Integrated Support Programme for Arable Agricultural Development (ISPAAD), National Agricultural Master Plan for Arable Agriculture Development (NAMPAAD) and the Zambezi Integrated Agro- Commercial Development project which are aimed at enabling self-sufficiency in grain crops. The expected increase in the area under production is likely to increase the incidence and spread of diseases, hence the need to identify and document the causal agents of such disease problems in the area.

The Botswana biosecurity pilot project in the Chobe district that was launched in November 2012 is also one of the primary reasons that require documentation of diseases that are found in the area, so that measures could be put in place to eradicate or avoid any introduction of new ones. Biosecurity systems are primarily concerned with preventing, controlling or managing hazards to life and health (FAO, 2007). Conservation tillage is also one of the main practice in Pandamatenga farms, which is done to reduce cultivation costs, to utilise their fertilizer in the same rows for consecutive years through precision tillage. It is also carried out as Muir (2012) defines it as basically any system of cultivating that reduces soil or water loss when compared to conventional mouldboard ploughing, which turns over the soil completely. According to (Muir, 2012), for pathogens such as fungi and bacteria conventional till buries crop residue which destroys many fungal and bacterial pathogens. Many pathogens use residue as an overwintering place, but are destroyed (or rendered incapable of causing damage) when they are buried. Hence, under conservation tillage, severity of some diseases can increase, potentially requiring more use of chemicals.

1.3.0 Objectives

The main objective of the study was to identify and characterize the pathogen(s) causing sorghum leaf blight (SLB) in Pandamatenga.

The specific objectives of the study were to:

1.3.1 assess the incidence and severity of SLB in Pandamatenga farms during the2012/13 growing season;

1.3.2 isolate and identify the pathogen(s) causing sorghum leaf blight (SLB) in Pandamatenga farms;

1.3.3 determine the survival of the pathogen(s) in stubble on the soil surface, and buried in the soil at depths of 15cm and 30cm;

1.3.4 evaluate the response of thirteen (13) different varieties of sorghum grown in Pandamatenga to inoculation by the SLB pathogen under greenhouse condition;

1.3.5 evaluate the in-vitro efficacy of three (3) fungicides commonly used by Pandamatenga farmers on the SLB pathogen.

Chapter 2.0 LITERATURE REVIEW

2.1.0 Growth requirements for sorghum

The optimum growth requirements of sorghum plants are a deep well drained fertile soil, Sorghum is best adapted to areas having an average annual rainfall between 450 to 650 mm which is fairly distributed during the growing season, temperate to warm weather (20- 30°C) and a frost free period of approximately 120-140 days (du Plessis, 2008). Sorghum is mainly grown on low potential, shallow soils with high clay content, which usually are not suitable for the production of maize. It is a warm weather crop which requires high temperature for good germination and growth. The minimum temperature for germination varies from 7 to 10°C.It is best planted when there is sufficient moisture and the soil temperature is 15°C or higher at a depth of 10 cm and the optimum temperatures for growth and development are 27- 30°C (du Plessis, 2008).

2.2.0 Diseases of sorghum

Sorghum is distinguished among other cereals by its unusually broad range of biotic and abiotic diseases because of the diversity of its uses and range of environments in which it is cultivated. In areas where the sorghum is traditionally grown, plants maybe attacked by as many as five or six foliar pathogens and an array of soil borne organisms. Foliar pathogens are very likely to produce the same symptoms, making disease diagnosis very important (Frederiksen, 1986). Further, variations that are found in height, vigour, pigment and other morphological characteristics affect disease expression thus complicating accurate diagnosis.

Sorghum foliar diseases such as leaf spots, blights, and other diseases which destroy leaf tissues or cause defoliation reduce photosynthetic leaf area and photosynthesis resulting in final grain yield loss (Agrios, 2005). However, assessment of yield loss due to loss of

photosynthetic area in southern Africa is difficult due to lack of statistical data on yield and yield losses (de Milliano, 1992).

The major sorghum foliar diseases and their pathogens are leaf blight (*Exserohilum turcicum*), target leaf spot (*Bipolaris sorghicola*), grey leaf spot (*Cercospora sorghi*), ladder spot (*C. fusimaculans*), rust (*Puccinia purpurea*), Zonate leaf spot (*Gloecercospora sorghi*), sooty stripe (*Ramulispori sorghi*), leaf anthracnose (*Colletotrichum graminicola*), rough leaf spot (*Aschochyta sorghina*), and tar spot (*Phyllachora sacchari*) (Odvody and Hepperly, 1992). Other diseases that affect the leaf sheath are southern sclerotial rot (*Sclerotium rolfsii*), banded leaf and sheath blight (*Rhizoctonia spp*), zonate leaf spot on sheaths, *Alternaria* spp, *Cochliobolus* spp and *Pyrenospora* spp of cereals and grasses also occur throughout the world (Agrios, 2005). *Exserohilum turcicum* has been reported as an important leaf disease in sorghum both in south, east and western African countries where sorghum is grown (de Milliano, 1992; Hulluka and Esele, 1992; Thomas, 1992).

Sorghum is attacked by a wide range of stem, leaf, and panicle diseases, but good levels of resistance to most or all of them are available. The reaction of the sorghum plant to diseases is to produce characteristic discolouration and colour patterns. The detailed expression of these is modified by the genetic make-up of the plant, especially by the plant colour factors. Shades of tan, red, or purple are produced, depending less upon the disease than upon the sorghum genotype (Doggett, 1988).

Some diseases can be recognized from symptoms alone especially where the characteristic appearance of the pathogen, as in mildew, rust and smut diseases, is an integral part of the symptoms or where the observer is very familiar with the disease. Even then, identification of the species involved may require microscopy of the sporulating structures (Waller *et al.*, 2002). Confirmation of the presence of a pathogen in a diseased plant is an essential step in

the diagnosis of disease and this often requires procedures of detection and isolation. There are many situations where ill-health of plants may not be clearly caused by obvious biotic agents therefore identification is key to a possible pathogen nomenclature, and correct management practices.

A study conducted by Mansuetus, (1995) in Tanzania in 1990 identified grain mould, grey leaf spot, anthracnose, leaf rust, leaf blight and covered kernel smut as major constraints to sorghum production. A list of sorghum diseases and their causative agents in Tanzania which may be representative of the diseases of sorghum in eastern and southern Africa is presented in **Table 2.**

Table 2 - Sorghum fungal diseases and their causal agents as identified at different locations in tanzania between1986 and 1990.

Disease	Causal organism
Acremonium wilt	*Acremonium spp*
Anthracnose	*Colletotricum graminicola*
Charcoal rot	*Macrophomina phaseolina*
Downy mildew	*Peronosclerospora sorghi*
Ergot	*Claviceps africana*
Grey leaf spot	*Cercospora sorghi*
Ladder leaf spot	*Cercopspora fusimaculans*
Leaf blight	*Exserohilum turcicum*
Leaf spot	*Phoma sorghina*
Leaf spot	*Mycosphaerella spp*
Rough leaf spot	*Aschochyta sorghina*
Leaf rust	*Puccinia purpurea*
Sooty stripe	*Ramulispora sorghi*
Stalk rot	*Fusarium moniliforme*
Headsmut	*Sporisorium reilianum*
Covered kernel smut	*Sporisorium sorghi*
Longsmut	*Tolyposporium ehrenbergii*
Loose kernel smut	*Sphacelotheca crueta*
Zonate leaf spot	*Gloecocercospora sorghi*

Source; (Mansuetus, 1995).

Frederiksen (1986) reported that three bacterial leaf diseases are common in sorghum and these are; bacterial leaf stripe, caused by *Pseudomonas andropogonii*; bacterial leaf streak, caused by *Xanthomonas campestris* pv. *holcicola* and bacterial leaf spot caused by *P. syringae* pv. *syringae*. Of the three, bacterial leaf stripe is more frequently reported in the tropical and sub-tropical regions, and the other two are more common on sorghum grown in temperate areas and at higher elevations. Some of the bacterial pathogens that can cause confusing symptoms in sorghum are *Pseudomonas andropogonii and Xanthomonas holcicola* making reliance on symptoms of little or no importance.

Important foliar fungal pathogens of sorghum of the world include *Exserohilum turcicum* (formerly *Helminthosporium turcicum), Peronosclerospora sorghi (Sclerospora sorghi), Sclerophthora macrospora, Ramulispora sorghi, Colletotricum graminicola, Cercospora sorghi* amongst others (Doggett, 1988; de Milliano *et al.*, 1992).

Foliar diseases are among the most common and recognisable diseases of sorghum. Extensive knowledge of some diseases and their causal agents is on record, and only a minimal amount is available on others (Odvody and Hepperly, 1992) which leaves room for further research in the field. Most foliar diseases of sorghum have very distinct symptoms especially in mature lesions. Factors like multiple diseases of sorghum on the same leaf, host pigment reactions, and other complex situations are said to obscure otherwise typical symptoms (Fox, 1993). Different pathogens can cause the same symptoms with very small difference that can only be realised through experience and or diagnosis.

2.3.0 Leaf blight of sorghum

Leaf blight of sorghum is wide spread in many humid areas where sorghum is grown. If the blight is established on susceptible cultivars before seed emergence, grain yield losses maybe

up to 50-70%. If infection is moderate or delayed until after the formation of the inflorescence, yield losses are minimal. (http://www.icrisat.org). understanding of sound management practices for the disease the complex of leaf blight and its pathogen is crucial in order to dev blights is very crucial enabling that different methods be employed on trying to manage them hence an integrated approach.

Northern corn leaf blight (NLCB) caused by *Exserohilum turicum* (Leonard and Suggs) is one of the major corn diseases in most corn-growing areas of the world and may cause substantial reductions in yield (Bashan *et al.*, 1995). Leaf blight can predispose plants to stalk rots caused by other pathogens. *E. turcicum* attacks maize, sudan grass, Johnson grass, gama grass, and other teosinte. Host specific races of *E. turcicum* from maize and teosinte apparently do not infect sorghum, but some heterokaryon biotypes can. Disease development is favoured by moderate temperatures (18-27°C) and heavy dews during the growing season while dry weather retards disease development. If the disease is established on susceptible varieties before panicle emergence, losses in grain yield of up to 50% may occur (Frederiksen, 1986). The symptoms begin as small, dark, water-soaked areas, becoming irregular or elliptical, sometimes linear, brown then straw coloured or greyish, with red-purple or tan borders, often 4x10 cm or larger, coalescing and leading to death of leaves (Frederiksen, 1986).

Exserohilum turcicum has high genetic variability in terms of virulence; genetic structure and several races have been reported that attack corn (Muiru *et al.*, 2010). The high level of genetic variability may explain the occurrence of the disease in the different geographical localities and ability of the pathogen to infect most of the germplasm including resistant varieties. Ramathani (2010) further found that *E. turcicum* was more severe in maize than sorghum, which was attributed to physiological adaptation on maize than sorghum.

Leaf blight caused by *Exserohilum* sp has been reported on sorghum in Botswana in a study by de Milliano (1992) and Pathak (1991) and its distribution in the Pandamatenga area was reported by Mohale (2007). Studies on the biology of the leaf blight pathogen are important so that more information is made available for design of interventions through research. Variation in the pathogen i.e. the ability of the pathogen to exist as different strains makes it difficult to employ control strategies. Molecular genetic analyses have demonstrated variability at the DNA level for the pathogen so too is the production of monocerin which the pathogen uses as a virulence factor.

Other diseases of sorghum reported in Botswana include; anthracnose, covered kernel smuts, charcoal rot, downy mildew, ergots, head smut, long smut, loose smut, oval leaf spot, sooty stripe, and pathogens such as *A. alternata*, *Cladosporium* spp. *Nigrospora* spp. *Diplodia/ Phoma* spp (Pathak, 1991; Mohale, 2007). The studies of which revealed only the genus of pathogens found in the Pandamatenga farms, but could not identify their species. Sorghum anthracnose, caused by *Colletotrichum graminicola* is another leaf blight increasing in importance (Cardwell *et al.*, 1989). The pathogen *Colletotrichum graminicola* infects leaves, stalks, peduncles and panicles, including the grain (Ali and Warren, 1992). *Colletotricum graminicola* is a highly variable pathogen, and there is evidence of races among pathogen populations (Cardwell *et al.*, 1989). Ali and Warren (1992) further explained that variability in virulence among pathogen populations may pose a threat to sorghum production with grain losses of up to 50% having been reported. Cardwell *et al.* (1989) also reported that there was no evidence of seed transmission to the pathogen.

2.4.0 Epidemiological studies of sorghum leaf blights

When a pathogen spreads to and affects many individuals within a population over a relatively large area and within a relatively short time, this is referred to as an epidemic

(Madden *et al.*, 2008). Madden *et al.* (2008) further defined epidemiology as the study of diseases, in space and time with the objective to trace factors that are responsible for or contribute to their occurrence or more specifically the science of populations of pathogens in populations of host plants, and the disease resulting therefore under the influence of the environment and human interferences. Most epidemics are more or less localised and cause minor to moderate losses because they are kept in check either naturally or by chemical sprays and other control measures (Agrios, 2005). The chances of an epidemic are increased when susceptibility of the host and virulence of the pathogen are greater, as the environmental conditions approach the optimum level for pathogen growth, reproduction, and spread, and as the duration of all favourable combinations is prolonged, provided no human intervention occurs to reduce or stop the epidemic.

It is very important to understand the disease very well to ensure that it is controlled at its most critical times. Understanding of disease epidemiology can also provide information for design of the most appropriate integrated disease management (IDM) program. This is exemplified by the study of the behaviour of epidemics and the reaction of their components to weather factors and control measures (Kranz, 2003). It provides information on such micro factors (temperature, humidity) that are considered when planning a disease management programme. Furthermore through systems analysis, comparative epidemiology can be used to develop tactics and strategies for a more efficient, economic and sustainable diseases management programme.

Agrios, (2005) explained that for a disease to become significant in a field, and particularly if it is to spread over a large area and develop into a severe epidemic, the right combinations of environmental factors must occur either constantly or repeatedly, and at frequent intervals,

over a large area. Being able to forecast a plant disease epidemic is therefore intellectually stimulating and also an indication of the success of modelling. It is extremely useful to farmers in the practical management of crop disease. Disease forecasting allows the prediction of probable outbreaks or increases in intensity of disease and, therefore, allows us to determine whether when and where a particular management practice should be applied.

Ngugi *et al.* (2000) conducted a study on the Epidemiology of sorghum anthracnose (*Colletotricum sublineolum*) and leaf blight (*Exserohilum turcicum*) at Alupe in western Kenya from 1994 to 1996. It was found that leaf blight epidemics always started earlier than those of anthracnose, but exhibited lower disease severity at crop maturity.

Many pathogens survive as mycelia, spores, or sclerotia within sorghum host residues, or in soils (Odvody and Hepperly, 1992). Odvody and Hepperly (1992) stated that sclerotia also exist freely in soils and depending on the fungus, germinate to produce initial inoculum of mycelia or conidia. Furthermore, the initial inoculum of the foliar pathogens is disseminated primarily by wind, rain, soil splash and soil contact. Muiru *et al.* (2008) in a study conducted in Kenya reported that agro-ecological zones with moderate temperatures accompanied by reliable rainfall recorded higher turcicum leaf blight (TLB) disease incidence and severity than those zones characterized by dry conditions with low rainfall levels. The study also revealed that there were variations in pathogen morphology, pigmentation, growth rate and sporulation rate in different media when comparing isolates from different agro-ecological zones. Chang and Hwang (2002) further found that leaf position significantly affected severity with lower leaves being more severely infected than the upper leaves.

Tillage has been used for millennia to prepare the soil prior to sowing many of the annual grain crops. It involves applying power to break up and rearrange the entire topsoil structure. It has the primary aim of destroying weeds and pests but is also important for incorporating, redistributing or releasing nutrients and making the soil texture suitable for seed sowing, seed germination and for easy penetration of seedling roots. Bowden (2000) explained that although a few diseases are suppressed under reduced tillage, most diseases are favoured with more residues on the surface. The main way that reduced tillage affects disease is by increasing the amount of inoculum. Tillage reduces crop debris that serves as a refuge for many pathogens. It also destroys volunteer plants that serve as a reservoir of pathogens, such as viruses or rusts that require a living host.

Another way that tillage can affect diseases is through changes in the microenvironment. Reduced tillage, for example, tends to increase soil moisture and decrease soil temperature (Bowden, 2000). These changes suppress some diseases such as dryland foot rot or common root rot. There are numerous other changes in the soil bulk density, porosity, and microbial community that may affect diseases. Tillage can also affect the behavior of vectors that carry diseases. For instance, aphids that carry barley yellow dwarf virus are less likely to land in fields with abundant crop residue on the soil surface. The effect of tillage on subsequent disease severity depends heavily on the previous crop (Bowden, 2000). For example, crop debris from wheat contains numerous pathogens which can harm a subsequent wheat crop. Therefore, no-tilling wheat into wheat stubble often results in high disease severity. On the other hand, crop debris from legumes contains very few pathogens that can harm wheat. Therefore, no-tilling wheat into legume stubble poses little risk. Pathogen mobility affects the scale of tillage effects. Tillage has a strong localized effect on a non-mobile pathogen such as the take-all fungus (*Gaeumannomyces graminis*). Conversely, tillage may have little localized

effect on a mobile pathogen such as the scab fungus (*Fusarium spp*), but regional changes in tillage practices affect the scab inoculum level of an entire region

2.5.0 General Disease Diagnostics

Diagnosis is the process of determining the cause of a disease. It can be a long or short process depending on one's ability and the nature of the problem. Once the cause is known, an appropriate control strategy can be developed.

When a pathogen is found on a diseased plant, such a pathogen is identified by referring to special manuals and if the pathogen has been associated with such a disease, and the diagnostician is confident that no other causal agents are involved, then the diagnosis could be complete, (Agrios, 2005). If however, the pathogen found does not have any previous reports to support the finding, then Koch's postulate is followed to verify the hypothesis that the isolated pathogen is the cause of the disease.

Most nutritional disorders however produce characteristic symptoms on leaves, stem, or roots which can be used in diagnosis under field conditions. Different nutritional disorders, or attacks by pests and diseases, may sometimes produce rather similar visible symptoms hence this always requires laboratory analysis to make the correct diagnosis and prescribe the right remedy (Grundson *et al.*, 1987).

According to Frederiksen (1986), sorghum is subject to an unusually large number of leaf diseases. Diagnosis can be complicated by factors including multiple diseases in one leaf, environment, and the host maturity. Differences in reactions from different varieties may also complicate diagnosis, because the physiological reactions of any particular pathogen is conditioned by the type of plant pigment produced by the given sorghum line. It therefore suggests that different varieties may respond to the same foliar pathogen with purple, red or tan reactions. Ultimately, it means descriptions of foliar diseases based on colour maybe

inadequate and unreliable. It is fortunate that most foliar diseases are distinct and can be recognized with practice.

Certain leaf pathogens can exhibit similar symptoms; *Drechslera gigantean*, *Exserohilum longirostratum*, and *E. rostratum* all are *Helminthosporium*-like pathogens that incite leaf blight in cereals (Chandramohan *et al.*, 2002). Leaf blight caused by this *Helminthosporium*-like fungi affect a wide range of grasses with very diverse symptoms. Jardine (1998) found that leaf blight diseases are more prevalent during humid conditions and high temperatures. It is very critical to identify the pathogen causing leaf blight symptoms in sorghum in the Pandamatenga area so that an appropriate disease management plan can be devised.

Isolates of the same pathogen sometimes show significant difference at race level. Corn isolates and broom corn isolates of *E. turcicum* are independent on races i.e. corn isolates cannot infect broom corn and broom corn isolates cannot infect corn (Chang and Fan, 1986). When estimating DNA variability between isolates of different host specificity, different races and different geographic sources in maize, Johnsons grass and sorghum, race specific diagnostic patterns were not found (Abadi *et al.*, 1996). Although it has a wide host range, it has been reported that races of Johnsons grass do not infect maize, and similarly sorghum races were also not found to infect maize.

2.6.0 Management of foliar diseases of sorghum

From seedling to harvest, constant vigilance is required against every possible disease epidemic in order to grow a healthy crop to its maximum yield (Fox, 1993). While most diseases can reduce profits such as powdery mildew on sunflower, some may occasionally cause total ruin if detected too late such as leaf blight in sorghum. It is essential to detect the disease outbreak at the earliest stages so that it can be controlled. Control measures are therefore dependent on these detections; hence reports from neighbouring farms should

always trigger control measures to be started as early as before there are any signs or symptoms of diseases in the crop. The success in disease control is when all pertinent information regarding the crop and it's pathogens, the history of the disease in other years, varietal resistance to the diseases, the environmental conditions expected to prevail, locality, availability of materials, land, labour, and costs are taken into account when developing a control program. Usually an integrated approach is recommended because it takes care of all the diseases that affect the crop. If however, a specific disease is known then it can be controlled as identified (Agrios, 2005).

Diversification of cereal cropping systems with alternative crops, such as oilseed, pulse, and forage crops, furnishes producers with a range of agronomic and economic options. Crop diversification also improves management of plant diseases through manipulation of host factors such as crop and cultivar selection, interruption of disease cycles through crop rotation, fungicide application, and removal of weeds and volunteer crops canopy using tillage practices and stand density (Krupinsky *et al.*, 2002). According to Krupinsky *et al.* (2002) management practices such as seed treatment, date and rate of seeding, balanced fertility, control of weeds, field scouting, harvest management, and record keeping can be utilized to manage plant diseases.

Local epidemics of northern leaf blight caused by *E. turcicum* usually originate from conidia on infested residues (Adipala and Ogenga-Latigo, 1994) in maize, making it an important component of pathogen survival during off season period. In a study by Adipala and Ogenga-Latigo (1994); Takan *et al.*, (1994) it was reported that plots that were exposed to stubble exhibited different levels of infestation depending on the distance from the stubble. The infestation decreased as distance from the source increased. Sanitation which involves eliminating or reducing the amount of inoculum by removal of infected plant parts and plant

debris in the farm is therefore a very important tool in disease reduction especially for those pathogens that overwinter in crop residue (Walters, 2009). In a study by Shree and Luke (1982) on the capacity of *E. turcicum* (Pass) Leo and Suggs, to survive in sorghum crop residues in the field, it was found that the pathogen had poor survival in the soil as a saprotroph. It was found that alternate graminaceous hosts and low seed-borne nature of the fungus might be involved in causing the seedling blight.

On evaluating the efficacy of crop species mixtures for the management of sorghum anthracnose caused by *C. sublineolum* and leaf blight caused by *E. turcicum.* Ngugi *et al.* (2001) found that crop mixtures delayed the time when the first symptoms were observed. The approach can be best suited for small holder farmers who often can allow different crops to simultaneously grow together in the same field, allowing little or no use of machinery. Anthracnose, leaf blight and rust can both be managed through host plant resistance. Sharma *et al.* (2011) found that some sorghum accessions had some level of resistance to either of the above disease and some exhibiting multiple disease resistance which can be very useful in sorghum resistance breeding programs. Gwary *et al.* (2008) demonstrated the use of an integration of fungicides, crop varieties and date of sowing to manage sorghum anthracnose. The results revealed that all the three factors had an influence in reducing disease severity.

Cultural control such as deep ploughing can be effective and considered a sustainable alternative to plant disease management. It is also elevated by the continuing problems with fungicides and breakdown of host plant resistance, as well as increasing concern for the environment. The choice of the cultural practices is however dependent on the crop and the pathogen in question, although a combination of several appropriate cultural approaches can be used to address more than one pathogen at the same time (Walters, 2009). The most effective form of disease control must be for each crop plant to use its own form of inbuilt defence against infection such that farmers have freedom in other aspects of crop

management to pursue maximum economic returns in a sustainable farming system (Wallwork, 2009). Lance (2013) stated that sorghum leaf blight can be controlled by rotation to non-susceptible crops and foliar applications of recommended fungicides and planting resistant hybrids as the most effective management strategy.

Pandamatenga farmers reported that they used three fungicides which are Artea, Impact, and Propicon in a rotation. The three fungicides were found to be belonging to the same class of Triazole compounds are amongst the very effective fungicides against a wide range of fungal pathogens and are among the most recent developments (Tortora *et al.*, 2002; Agrios, 2005). The triazoles are very specific in their mode of action – they inhibit the biosynthesis of sterol, a critical component for the integrity of fungal cell membranes. Because their site of action is very specific, there are resistance concerns. Users of triazoles are therefore advised to rotate fungicide chemical families in their disease management programs, not simply rotate to a different member of the triazole family. Product formulations of the triazole fungicides include dry and liquid flowables, wettable powders, water soluble powders, and emulsifiable concentrates. Appendix 8 outlines diseases that they have been recommended to control by the three triazoles. Bowen and Pedersen (1988) conducted a study on effects of propiconazole on *Exserohilum turcicum* and found that it inhibited mycelial growth of *E. turcicum*, the causal agent of northern leaf blight, but did not inhibit conidial germination, while mancozeb inhibited only conidial growth of the same pathogen.

CHAPTER 3.0 MATERIALS AND METHODS

3.0.1 Description of Pandamatenga farms

The study was conducted in Pandamatenga farms (latitude 18°26' to 18°43', longitude 25°27' to 25°37' and altitude of 1069 meters above sea level) in the Chobe District of Botswana. The area experiences a semi–arid climate characterized by summer rainfall and annual average total rainfall of 450-500mm. Most of the rain falls between October and April, with December, January and February being the peak months (GoB, 2005). The total area of the farms is 25000ha divided into northern (16000 ha) and southern plain farms 9000ha) **(Appendix 8)**. The Northern plain is dominant in black cotton soils and the clay loam soils in the southern plains. Below is map that shows the Pandamatenga farms.

3.1.2 Assessment of incidence and severity of SLB in Pandamatenga during 2012/13 cropping season

3.1.2.1 Field selection, sampling, layout and disease assessment

The farms to be assessed were identified with the assistance of the Chairperson of the Pandamatenga Commercial Farmers Association (PCFA). A total of 8 farmers who were willing to participate in a survey were chosen. Individual farms were visited to discuss the history of the farm and its management.

There was a total of 25 farms in Pandamatenga each farm measuring 500ha which are all enclosed in a perimeter fence of about eighty four (84) kilometres to keep away wild animals. A total of 13 farms were used in this study. Each farm had access routes at the peripheries and in the centre of the farms. These access routes were used as a guide to select the sampling stations to avoid damage to farmer's crops **(Figure 1)**. To assess for disease incidence and severity, five (5) sampling areas were identified to represent all four sides and the centre of

the field.

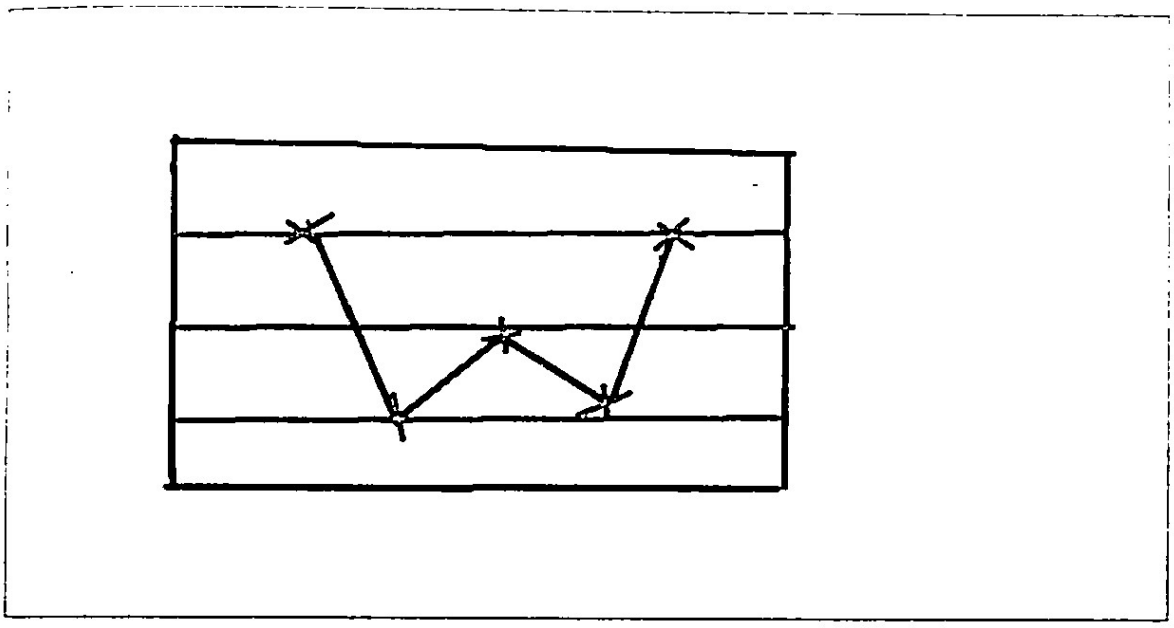


Figure 1- An illustration of a farm showing the sampling stations with letter W

From one corner of the field a car was driven along the length of the farm for 100m then from the periphery six (6) rows were counted into the field. Each row consisted of eight lines of sorghum plants separated by a spray route. Six (6) rows were counted making it 50 metres to the inside of the field and characterised as a sampling area. The route that runs through the centre was used to drive about 1 km (half way the length) into the field where one (1) sampling area was assessed. The other 4 points were towards the peripheries of the farms as illustrated in **Figure 1.**

To assess incidence and severity at each sampling area, three (3) stations measuring 1m x 1m were randomly determined by throwing a 1 m^2 quadrat for sorghum below knee height or measured using a measuring tape for mature and bushy crops. In each sampling station with about twenty (20) plants in a quadrat, ten (10) plants were chosen at random and assessed for both incidence and severity. Disease incidence was calculated by counting number of plants that were showing disease symptoms (*x)* out of the ten (10) plants per station. The figure was therefore calculated into percentage (*x*/10*100) and the average % of the 5sampling areas

(replications) were regarded as the incidence for one (1) farm. Severity on the other hand was the proportion of the leaf area that was showing symptoms per plant. The symptoms were rated using the leaf blight rating scale of Aden (1991) **(Table 3)** and a pictorial guide to the ratings is in **Appendix 6.**

3.1.2.2 Experimental design and data analysis

For the survey of disease incidence and severity a Completely Randomized Design was used. The treatments were the sampled fields and the replications were the sampling areas. Data on incidence and severity was converted into percentage and transformed using the arcsine square root [arcsine$\sqrt{}$ (percent x/100)] were subjected to non-parametric multiple comparisons Kruskal-Wallis test at $p = 0.05$

Table 3- Disease scoring: 1-5 leaf blight rating scale

Rating value	**% leaf area infected**	**Description**
1	0	Free from disease
2	<10	Slight symptoms
3	11-25	Moderate symptoms
4	26-40	Moderately severe symptoms
5	>40	Very severe

3.1.3 Isolation and identification of the pathogen(s) causing sorghum leaf blight (SLB) in Pandamatenga

3.1.3.1 Collection of samples for identification of pathogens

Diseased leaf samples from MR-Buster, PANNAR 8909, Tiger and Enforcer were collected and placed into plastic bags of about 15cm x 30cm in size, so that the leaves maintained their shape until processing. The collected samples were stored in a cooler box with ice for three days during field surveys and transferred to a refrigerator immediately on arrival in the laboratory at the Botswana College of Agriculture in order to retain their freshness for identification. All samples were examined within 5 days from the day of collection. The survey was carried out from the 10^{th} -14^{th} January 2013. The survey was only conducted once due to limitation of resources.

3.1.3.2 Isolation of fungi from infected leaves and spore extraction

To isolate the pathogens, pieces of leaves ($1cm^2$) with lesions were cut and surface disinfected in 70% ethanol for 1 minute then transferred into 15% hydrogen peroxide for 1 minute and kept in 70% ethanol for 1 minute. Thereafter the pieces were washed in 10 changes of sterile distilled water. The pieces were transferred to a sterile paper towel in a petri dish to remove excess water (Sharma and Pandey, 2010). Finally the pieces were aseptically grown on Water Agar (WA) plates each containing four pieces. WA was used because it minimises bacterial contamination and to enable sporulation (Watanabe, 2010). To obtain single spore pure cultures, individual spores were picked from WA plates using a needle and placed on Potato Dextrose Agar (PDA) (Waller *et al*,. 1998). The inoculated plates were placed in an incubator at 25°C with 12 hours of light followed by 12 hours of darkness for 48 hours. Pure cultures were maintained in WA at 5°C for future inoculations. To extract the spores from the media for further inoculations the following procedure was used; a 1500 ml conical flask was sterilised in an autoclave for fifteen (15) minutes. The

colonised culture medium was then transferred into the conical flask with 800 ml sterile distilled water. This was agitated using a Stuart orbital flask shaker, at 250rpm for forty five (45) minutes to extract as much spores from the medium as possible. The supernatant was then collected and mixed with 0.01% tween 20 surfactant (Aden, 1991) and again agitated in a shaker for ten (10) minutes at 200rpm.

Before inoculating the plants with conidia the concentration of the inoculum was standardized (Lombard, 1998). The direct counting method used in this experiment is described below:

A petri dish was graduated into 10mm* 10mm fields and 10 ml of the inoculum concentration was poured inside. The area of field of view under the microscope was designated Zmm^2. If on Z mm^2 are X cells (X-mean of cell counts/field therefore $100mm^2$ are $(X*100/Z\ mm^2) = Y$ cells

If 0.1 ml produces Y*10 cells then 10ml will produce Y*1000 cells, therefore the spore count used was;

25*100/ 33.33 = 75 cells

Then $7.5x\ 10^3$ spores per ml were used to spray the plants.

This method was used as there was no access to a haemocytometer for counting the spores.

3.1.3.3 Identification of isolates of fungal pathogens from leaves

Using a dissecting microscope individual spores and hyphae were picked from the media using a needle and mounted on a microscope slide and observed under a light compound microscope. The morphological characteristics of spores and conidiophores of the fungus were recorded. The characteristics of 20 conidia and conidiophores recorded were septation, length and width. Species confirmation was based on morphological characteristics and sporangial morphometrics. The spores from the isolates were further used to inoculate the test plants on 3.3.0.

3.1.4 Comparison of Water agar (WA), V8-juice agar, Potato dextrose agar (PDA) as media for growth and inoculum production of SLB pathogen under laboratory and incubator conditions

Production of inoculum on artificial media is always important for laboratory work and for inoculation of plants in the field. To produce abundant conidial inoculum for inoculation of host plants or pathogen identification and other tests it is necessary to determine the media and temperature for growth of the pathogen under study. The objective of the experiment was to assess the effect of WA, V8-juice agar and PDA on growth and sporulation of the SLB pathogen. The three media were prepared as described below.

3.1.4.1 Preparation of media

The three media were prepared following standard recipes as detailed in the **Table 4**. Five hundred millilitres of each medium were sterilised in an autoclave for 15min at 2000 pascals (pa) and aseptically poured onto petri dishes in a laminar flow cabinet. The plates were then allowed to solidify for 4-5 hours before inoculation.

Table 4 - Recipes three media PDA, WA and V8- juice agar

Name of medium	Contents	Quantities	Reference
*Potato Dextrose Agar	distilled water	400ml	
(PDA)	PDA	15g	
**Water Agar (WA)	distilled water	400ml	
	bacteriological agar	7.5g	
***V8 juice agar	distilled water	400ml	
	V8 juice	100ml	
	$CaCO_3$	2g	

Sources: *www.sc.mahidol.ac.th, **www.bionet/bionet/mm/mycology, ***http://bugs.bio.usyd.edu.au

To assess the suitability of each medium as sporulation medium, 1 cm^2 WA disks were aseptically cut from margins of a highly sporulating fourteen (14) day- old culture using a flame-sterilised cork borer. The disks were placed at the centre of each petri dish for the three different media. The inoculated plates were incubated at room temperature averaging 26°C and in an incubator at constant temperature of 25°C with alternating twelve (12) hours of light and 12 hours of darkness. The plates were placed in transparent tumbler which was sealed with its plastic lid, and it was placed western window of the laboratory to maximize exposure to sunlight. The room temperature was highly dependent on the weather conditions prevailing in summer.

3.1.4.2 Experimental design and Data collection and analysis

For each medium (Treatment), three (3) replicate plates were used for the two incubation conditions. The experimental design was a 2x3x5x3 split plot with incubation conditions as the main plots, media as subplots and sampling time as sub subplots with 3 replications (2 x Incubation conditions, 3x media, 5 x sampling times, and 3 x replications). The data collected were colony diameter and degree of sporulation at twenty four (24) hour intervals for one hundred and sixty eight (168) hours. Presence and absence of Sporulation was determined by observing the petri dishes under a dissecting microscope and recording the result in the process. The data were subjected to analysis of variance (ANOVA) using SAS version 6.2, proc mixed procedure and if f-values were significant, means were separated using LSD test at P= 0.05.

3.2.0 Survival of SLB Pathogen on sorghum stubble on the surface and buried at 15 cm and 30 cm depths

The objective of this experiment was to determine the survival of the SLB pathogen that may come as a result of different tillage practices. The experiment was conducted at the Botswana College of Agriculture farms in a plot that was previously planted with cowpeas. The trial

was conducted from March to May 2013. It was assumed that the experimental plot was free from turcicum leaf blight of sorghum.

3.2.1 Preparation of holes

Two holes measuring 50cm by 50cm and one meter apart were dug. The first one was fifteen (15) cm while the second was 30cm deep. The holes were first treated with Chlorpyrifos (Organophosphate) 480g/l, registered by Agro-Serve (Pty) Ltd trading as EFEKTO South Africa, for control of termites to avoid their damage to the buried stubble. The holes were therefore left for 24 hours before the stubble was buried.

3.2.2 Collection and preparation of sorghum stubble

Sorghum stubble pieces were collected from sorghum varieties that have been planted at the Botswana College of Agriculture farms during the 2011/2012 cropping season. Sorghum stubble was stored in perforated plastic bags in the field. The sorghum stalks were cut into pieces 5-10 cm long using a knife. Fifteen (15) kg of stubble was weighed and spread on the surface of a polyethylene plastic bag to reduce contact with the soil. It was disinfested by spraying with absolute ethanol until it was moist and left to dry for three hours. The spraying of spores was just superficial infestation as opposed to assuming that the pathogen had time to "colonise" the stubble. After three hours the stubble was infested with the pathogen by spraying with 500ml spore suspension of *E. turcicum* at a concentration of 6.5 $x10^3$ spores/ml and allowed to dry for another three hours. The inoculated stubble was packaged into nine 1.5 kg perforated plastic bags. Ten (10) pieces of stubble were collected from each treatment on a weekly basis for a period of five weeks. The samples were taken to the laboratory for analysis. In the laboratory, individual pieces of stubble were washed with two washes of sterile water and incubated in a moist chamber at room temperature. The moist chamber was prepared with a 30cm x 20cm opaque plastic container (tumbler) with a clear lid. Plastic tumbler with sterile paper towel laid at the bottom and closed tightly with a lid. The stubble

pieces were incubated for 48hrs in the laboratory near a window with natural light and thereafter observed under a microscope for fungal growth and sporulation. The stubble pieces with *E. turcicum* conidia were counted per replication and any other pathogens that were found were identified.

3.2.3 Experimental design and data collection

The experiment was conducted using a Completely Randomised Design (CRD).

Three bags (replications) were exposed to each treatment, surface, 15cm and 30cm depths with each plastic bag placed in its hole. The holes were one metre apart.

The data were subjected to Analysis of variance and treatment means compared using LSD test at p= 0.05

3.3.0 Response of MR-Buster and twelve (12) other sorghum varieties on SLB pathogen under greenhouse conditions.

3.3.1 Sowing of Sorghum test plants

Thirteen (13) different varieties of sorghum were used in the experiment including MR-Buster which the pathogen was isolated from. These were, Segaolane, Sephala, BSH 1 (supplied by the Seed Multiplication Unit, Department of Agricultural Research) and PANNAR 8706, PANNAR 8909, PANNAR 8816 (supplied by Pannar seed (Pty) Ltd), Tiger, NUS 510, NUS 421, NUS 456, (supplied by Nuseed (Pty) Ltd), NS 5655 (supplied by Capstone seeds South Africa), MR-Buster, MR-Taurus (supplied by pacific seed) (obtained from Pandamatenga farmers who purchase most of the seeds they plant from the Botswana Agricultural Marketing Board (BAMB) which imports most of the seeds they sell from South Africa).

The thirteen (13) sorghum varieties (treatments) were grown in a greenhouse. Each variety was sown in three, eighteen (18) cm diameter black plastic bags (replication) containing 4kg

potting soil (Trademark, Gaborone) at a rate of ten (10) seeds per pot. Later at four leaves stage they were thinned to five plants.

The crops were watered with 500ml of water per pot twice per week. Water was sprinkled on plants leaves in the greenhouse for ten (10) minutes daily maintaining a very humid environment for optimum growth of the crops and the pathogen. A hosepipe with an adjustable nozzle was used to sprinkle water on the plant's leaves, to increase the humidity in the greenhouse.

3.3.2 Preparation of inoculum and inoculation of test plants

Twenty (20) day-old V8- juice cultures of the SLB pathogen isolated from sorghum variety MR-Buster, were used to extract spores for inoculation. Spore suspension was prepared using the spores obtained from **3.1.3.2.**

Forty five (45) days after sowing, sorghum test plants were inoculated by spraying each plant with ten (10) ml of the spore suspension using a spray bottle (Aden, 1991).The plants were covered with clear plastic bags for forty eight (48) hours to maintain high humidity for the fungus to grow. High humidity was also maintained in the greenhouse by wetting the house surfaces after every twelve (12) hours to keep the place moist for the first week. Afterwards the plants were sprinkled with water on the leaf surface daily to enable fungal growth. The first disease score of all the treatments was made forty eight (48) hours after removing the plastic cover. Disease scoring was done using the leaf blight scoring scale by Aden (1991).

3.3.3 Experimental design and Data collection and analysis

The experimental design was a Completely Randomised Design (CRD) with varieties being the experimental treatments, and units and parameters number of lesions per leave, number of plants infected, with each variety replicated three times.

The data collected over a five week period were: number of days to symptom development symptoms, average number of lesions of five tagged plants per bag and number of the tagged plants showing symptoms for the first time. Data was exposed to ANOVA to establish if there is any difference in pathogenicity amongst the varieties. Significant means were separated using the LSD test at p=0.05 to determine the level of significance.

3.4.0 Evaluation of in-vitro efficacy of Impact (Flutriafol 125g/l), Propicon 250 EC (propiconazole 250g/l) and Artea (propiconazole 250g/l and cyproconazole 80g/l) in controlling SLB pathogen.

Three triazole fungicides: Impact (Flutriafol 125g/l), propicon 250 EC (propiconazole 250g/l) and Artea (propiconazole 250g/l and cyprocanazole 80g/l) were found to be commonly used by farmers in Pandamatenga were selected for evaluation.

Two experiments were conducted to test the fungicidal and fungistatic effects of the three fungicides. Fungicidal effect was assessed to check if the fungicides kill a growing fungal culture while the fungistatic effect was to assess if the fungicide prevent spore germination and colony growth.

3.4.1 Evaluating fungicides using paper discs soaked in fungicide suspension on V8- juice agar and a mixture of fungicide +V8-juice agar

Two experiments were conducted to evaluate three fungicides for their efficacy. Five day-old pathogen cultures grown on V8-juice agar were used for both experiments. In the first experiment, filter paper disks of ten (10) mm diameter were soaked in the fungicide suspensions as recommended by the label for the control of blights. Two discs of each fungicide were placed at the margins of a fifteen (15) mm actively growing fungal culture in each petri dish. The petri dishes were incubated at room temperature in the laboratory near a west facing window. In the second experiment, fungicides were tested using V8- juice agar

mixed with fungicide suspension for their efficacy in controlling *E. turcicum*. In this experiment 600ml of molten V8- juice agar was prepared as described in 3.1.4.1. It was then divided into three 200ml glass bottles. Each fungicide was calibrated for 200ml media by calculating how much of the chemical should be mixed with 200ml of water. Each fungicide (treatment) was pipetted into each bottle of molten media and shaken until a uniform suspension was observed. The mixtures were stirred using a magnetic stirrer until a uniform colour was obtained poured into petri dish and left to set for twenty four (24) hrs. Five petri dishes per mixture were inoculated with the pure culture spores of the pathogen in the form of an agar block and left in the laboratory next to a west facing window for provision of light.

3.4.2 Experimental design and data collection and analysis

A Completely Randomised Design (CRD) was adopted for both experiments. Three fungicides each with five plates were used as replications for both trials. Diameter of mycelium clearance under each disk was measured daily for five days using a ruler. The data were subjected to ANOVA and if f-value was significant (p=0.05) the means were separated using LSD test to establish the difference in efficacy of the three fungicides. The diameter for mycelium was measured daily for five days using a ruler The Data were subjected ANOVA and significant means separated using LSD test at p=0.05.

Chapter 4.0 Results

4.1.0 General Field observations at Pandamatenga farms

4.1.1 Meteorological data

Monthly rainfall received during the season under study from October 2012 to April 2013 ranged from a low of 2.8mm in October 2012 to a high of 211.5mm in January 2013 making a cumulative total rainfall of 335.5mm for the whole season (**Appendix 3**). The season was also characterised by humidity ranges of 49% in October 2012 to a high of 86% in January and February 2013.

4.1.2 Agronomy and farming system at Pandamatenga farms

The overall soil conditions in Pandamatenga farms are vertisols very rich in clay. Grain crops are the mostly produced among the farms, the crops include sorghum and sunflower, the common of which is sorghum (**Table 5**). Most farms practiced winter ploughing and a few farms did not (farms Q6, Q9, Q12, and Q14). Winter ploughing contributes to moisture retention of a soil and also interferes with the life cycle of most soil pathogens. Fungicides were also used to control diseases by all the farms except Q12. Mono cropping was found to be the predominant practice amongst the farms as compared to crop rotation. The crops were also their advanced stages of growth from flag leaf to flowering.

Table 5 - General information about each farm collected from farmers during SLB survey in March 2013

Field No.	Location (NP/ SP)	Soil types	Sorghum variety	General crop husbandry	Previous season crop	Growth stages
Q1	NP	Vertisols	MR-Buster	Wp, f	Sorghum	Booting
Q6	NP	Vertisols	PANNAR 2566	NWp, f	Sorghum	Booting
Q9	NP	Vertisols	MR-Buster	NWp, f	Sorghum	Booting
Q12	NP	Vertisols	MR-Buster	NWp, Nf	Sorghum	Flowering
Q14	NP	Vertisols	Enforcer	NWp, f	Sorghum	Booting
Q17	NP	Vertisols	PANNAR 8909/Tiger	Wp, f	Sunflower	Flowering
Q18	NP	Vertisols	MR-Buster	Wp, f	Sunflower	Flowering
Q26	NP	Vertisols	PANNAR 8909	Wp, f	Sunflower	Flag leaf
Q28	NP	Vertisols	MR-Buster	Wp, f	Sunflower	Flag leaf
Q30	NP	Vertisols	MR-Buster/ Enforcer	Wp, f	Sunflower	Booting
Q38	SP	Clay loam	MR-Buster	Wp, f	Sorghum	Flag leaf
Q41	SP	Clay loam	MR-Buster/ PANNAR 8908	Wp, f	Sorghum	Booting
Q109	SP	Vertisols	MR-Buster	Vs	Nil	Booting

Wp: winter ploughing, NWp: no winter ploughing, f: fungicides, Nf: no fungicides, Vs: Virgin soils, NP: northern plains, SP: southern plains, Nil: no crop grown previous season Gs: Growth stage

4.2.0 Disease incidence and severity in Pandamatenga farms during the 2012/13 cropping season

4.2.1 Disease incidence

Sorghum leaf blight was found to occur on sorghum variety MR-Buster, Pannar and Enforcer in all the sampled fields in the Pandamatenga commercial farms. Disease incidence was found to be from a low of 2% in farms Q28 and 38 and maximum of 100% in farms Q9, 12 and 14 (**Table 6**). The highest disease incidence was recorded in the northern plains on variety MR-Buster (**Appendix 6**), where the fields had a sorghum crop planted in the previous season. In the southern plains the highest incidence was on variety MR-Buster in Q41 with 76%, and the farm also had sorghum the previous year.

Table 6 - Leaf blight incidence in Pandamatenga commercial farms during the 2012/13 ploughing season

Field No.	Location (NP/ SP)	Sorghum variety	Disease incidence (%)
Q1	NP	MR-Buster	98 ± 2.00a
Q6	NP	PANNAR 2566	50 ± 7.07c
Q9	NP	MR-Buster	100 ± 0.00a
Q12	NP	MR-Buster	100 ± 0.00a
Q14	NP	Enforcer	100 ± 0.00a
Q17	NP	PANNAR 8909	98 ± 2.00a
Q18	NP	MR-Buster	72 ± 5.83b
Q26	NP	PANNAR 8909	4 ± 2.45e
Q28	NP	MR-Buster	2 ± 2.00e
Q30	NP	MR-Buster	14 ± 2.45d
Q38	SP	MR-Buster	2 ± 2.00e
Q41	SP	MR-Buster	76 ± 6.78b
Q109	SP	MR-Buster	6 ± 2.45ed
Mean			**55.57**
LSD (P≤ 0.05)			**9.67**
CV (%)			**13.73**

Means with the same letters are not significantly different p=0.05, LSD test, figures after incidence with plus or minus are error margins, NP: Northern plains SP: Southern plains

4.2.2 Sorghum leaf blight severity

Severity rating of SLB varied from a low of 1.20 in farms Q38 and 28 and a maximum of 4.40 in farm Q9 (**Table 7**). Disease severity in variety MR-Buster was found to vary in most farms with a rating ranging from 1.20 to 4.40. The other varieties generally showed low severity when compared to MR-Buster.

The farms in the northern plains had a relatively higher level of severity compared to the southern plains.

Table 7 - Leaf Blight severity in pandamatenga commercial farms during the 2012/13 ploughing season

Field No.	Location (NP/ SP)*1	Sorghum variety	Leaf area infected (mm)*2
Q1	NP	MR-Buster	1.20 ± 0.06e
Q6	NP	PANNAR 2566	2.20 ± 0.79c
Q9	NP	MR-Buster	4.40 ± 1.51a
Q12	NP	MR-Buster	3.60 ± 1.57a
Q14	NP	Enforcer	3.60 ± 1.51a
Q17	NP	PANNAR 8909	3.20 ± 1.57a
Q18	NP	MR-Buster	2.00 ± 1.51a
Q26	NP	PANNAR 8909	1.40 ± 1.02bc
Q28	NP	MR-Buster	1.20 ± 0.13de
Q30	NP	MR-Buster	2.00 ± 0.38d
Q38	SP	MR-Buster	1.20 ± 0.06e
Q41	SP	MR-Buster	2.20 ± 1.11b
Q109	SP	MR-Buster	1.60 ± 0.19de
CV			**16.05**
LSD (P≤ 0.05)			**0.31**
Pr>F			**<.0001**

*1 NP: Northern plains SP: Southern plains; *2 Disease severity rating scale: 1-5*3 Means with the same letters are not significantly different p=0.05 (Tukey Grouping), figures after incidence with plus or minus are error margins

4.3.0 Isolation of the pathogen causing leaf blight symptoms on sorghum

A total of four fungal isolates were purified from the thirteen (13) leaf samples. *Exserohilum turcicum* was found in all the thirteen leaf samples. Other fungi isolated were *Rhizopus sp* in four (4) leaf samples, *Alternaria sp* in three samples *and Aspergillus sp* in one sample (**Table 8**).

Table 8 - Fungal isolates cultured and identified from sorghum leaf samples collected from pandamatenga farms during the 2012/13 cropping season.

Isolate Number	**Identification**	**Number of leaf samples assessed**	**Number of leaf samples with fungal isolate**
1	*Exserohilum turcicum*	13	13
2	*Rhizopus sp*	13	4
3	*Aspergillus sp*	13	1
4	*Alternaria sp*	13	3

4.3.1 Morphology and identification of fungi associated with leaf blight symptoms on sorghum

Fungal isolate 1whichwas isolated from all 13 infected leaf samples was identified as *E. turcicum* because of the following morphological features:

a. sporulation on sorghum; the fungus readily formed conidia throughout the leaf surface resulting in a culture that was grey to brown in colour,
b. Conidial ontogeny and shape: the conidia appeared brown, tapering towards the point of attachment to the conidiophore; more than one conidia were also found to protrude at the end of each conidiophore **(Appendix 5)**.

c. Conidial septation: The conidia were septate with an average of six septa per conidium (**Figure 2 and 3**) leaf symptoms: The pathogen produced characteristic symptoms of *E. turcicum* (water soaked longitudinal lesions that turned brown after sometime) (**Appendix 5**).

Figure 2 – Conidiophores of *E. turcicum*

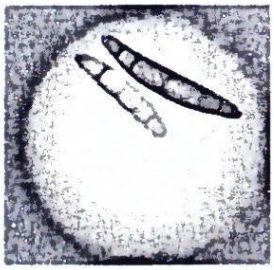


Figure 3 – Spores of *E. turcicum*

Isolate 2 was identified as *Rhizopus sp* because of the presence of stolons, aseptate hyphae, large sporangia with well-formed collumella and sporangiophores in clusters or singles supported by well-formed rhizoids.

Isolate 3 identified as *Aspergillus sp* was found in two cultures, it grows fast with a whitish mycelium and dense, erect conidiophores. The colony appeared velvety with a vigorous dense growth.

Isolate 4 identified as *Alternaria sp* was found in three cultures. It is fast growing, with greyish- black suede-like mycelium. Spores were also readily available within the first 24hours that enabled observation on the microscope and compared to literature for reference.

The most frequently isolated fungus in the infected leaves was *E. turcicum* and its description suggests that it is the cause of the SLB in the Pandamatenga area.

4.4.0 Determination of suitable growth media for E. turcicum growth under laboratory and incubator conditions

Exserohilum turcicum generally sporulated on all three tested media. Sporulation was heaviest on WA within the first 24hours of inoculation, followed by V8-juice agar and PDA was poorest with sporulation occurring only after 168hrs (**Table 9**). The difference in colony growth was not readily distinguishable in the first 24- 72 hours (**Figure 4 and 5**). The fungus produced a distinct dark brown pigment in V8-juice agar but little or no pigment in the other two media.

Figure 4 – Diameter of *E. turcicum* culture 24 hours after incubation in three different media

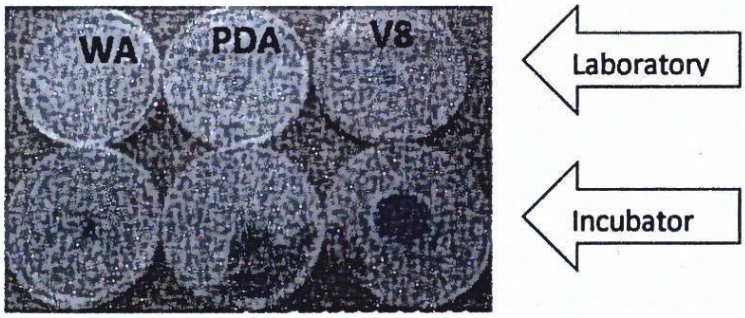
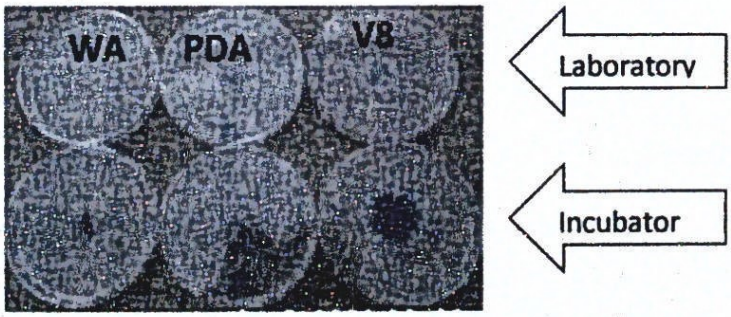


Figure 5 – Diameter of *E. turicicum* culture 72 hours after incubation in three different media



The pathogen was found to produce velvety mycelium, grey-black in colour, radially smooth spreading hyphal growth which tends to produce whitish margins on V8-juice agar (**Table 9**). The pathogen produced several conidia at the end of each conidiophore (**Appendix 4**).

Table 9 - Colony descriptions and sporulation of *E. turcicum*

Media type	Colony character		Reverse colour	Zonation	Spore type	Sporulation	Time to sporulation
	Texture	Surface Colour					
PDA	Velvety thick	Black hyphal growth	Grey	Radially smooth, spreading hyphal growth	Conidia	Poor	After 7 days
WA	Velvety fine	Greyish black	Grey	Radially smooth spreading greyish hyphae that can easily be distinguished	Conidia	Heavy	24 hours
V-8 juice agar	Velvety thick	Grey with white ring at the margins of growth	Dark brown ring-like	Radially spreading whitish grey margins	Conidia	Moderate	48 hours

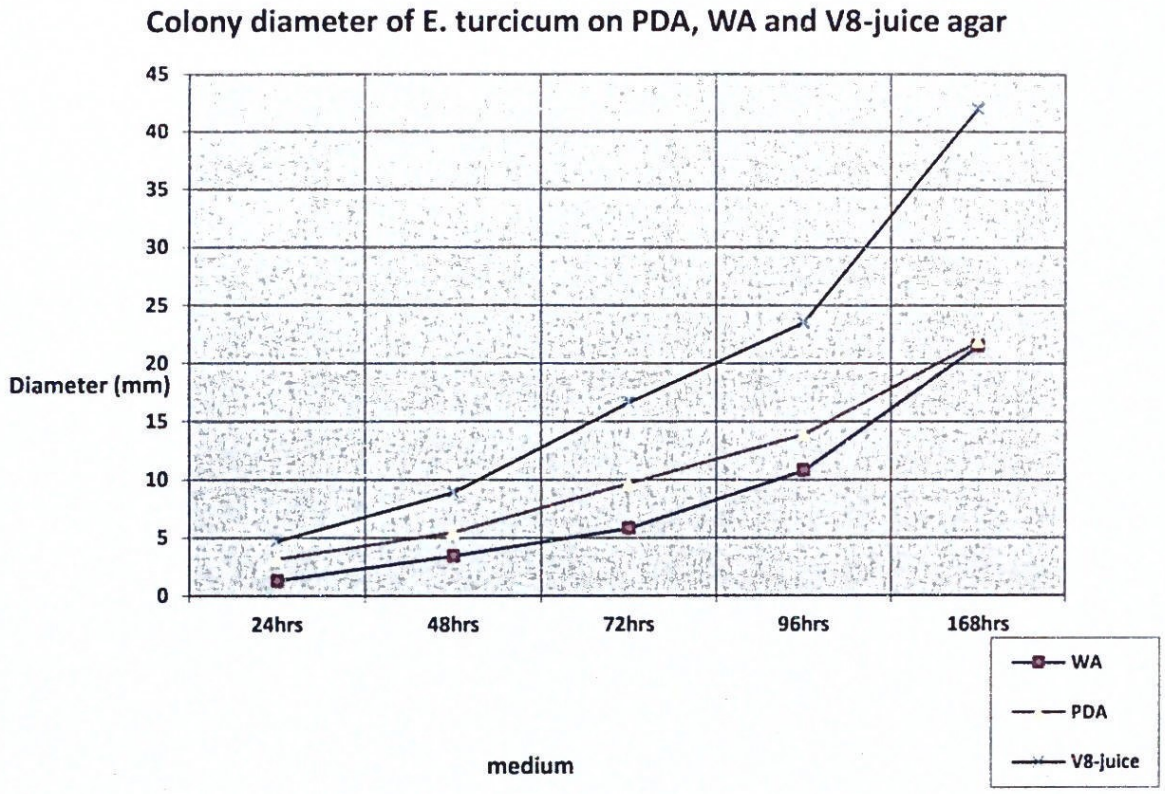
V8-juice agar exhibited the most fungal mycelial growth compared to WA and PDA both in the incubator and under laboratory conditions and WA was the least of the three with colony diameter of 1.25±0.70 mm after 168 hours (**Graph 1**). Cultures in the incubator generally developed faster than those under laboratory conditions (**Graph 2**). Growth on V8-juice agar was always faster than the other two media from the first 24 hours both in the laboratory and incubator. PDA growth was slower in the incubator in the first 24hrs then from 48 hours it became faster in the incubator (**Table 10**). WA on the contrary had the most growth in the laboratory at all the times as opposed to the incubator.

Table 10 - Colony diameter of *E. turcicum* on PDA, V8-juice agar and WA, exposed to an incubator and laboratory conditions measured for 168hrs

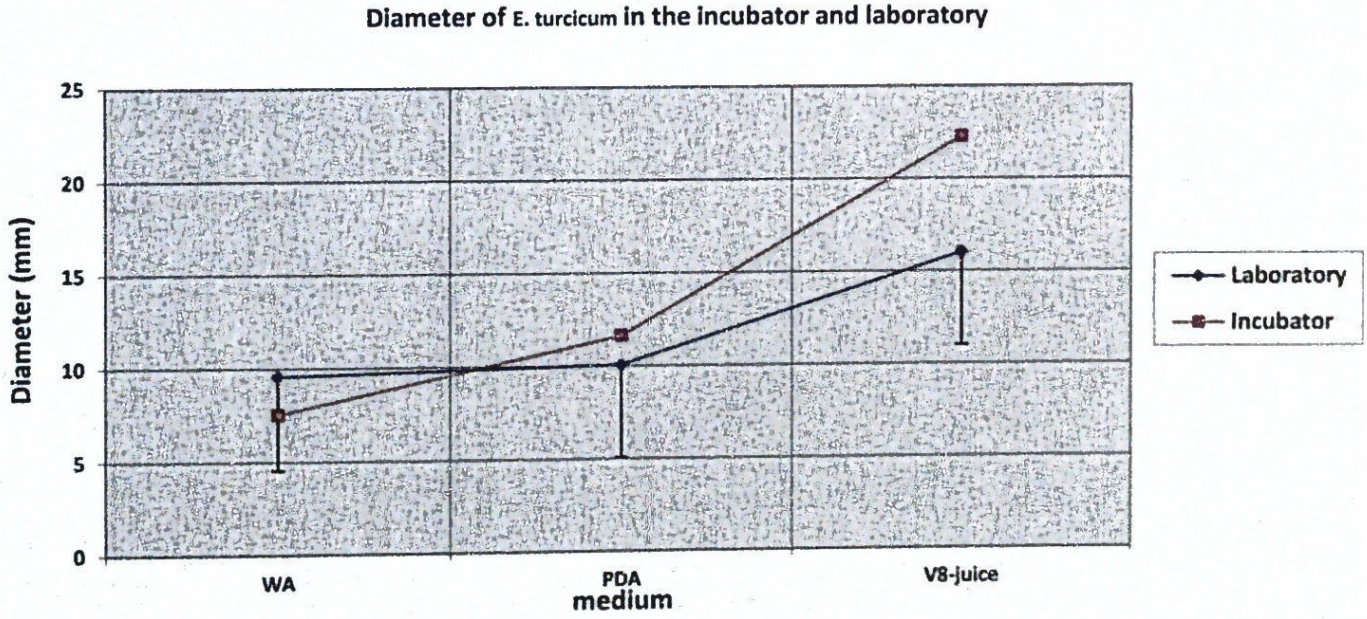
Time (hrs)	Condition	Mean colony diameter (mm) on different media		
		PDA	WA	V8-juice agar
24	Inc	3.12	0.25	5.63
	Lab	3.50	2.25	3.75
48	Inc	4.12	2.00	11.50
	Lab	6.75	4.75	6.75
72	Inc	11.00	4.00	21.25
	Lab	8.25	7.50	12.00
96	Inc	15.75	8.75	28.00
	Lab	12.00	12.75	19.00
168	Inc	24.00	22.50	45.00
	Lab	19.75	20.00	39.00
LSD ($p< 0.05$)		0.93		

*Means in a row compares diameter of *E. turcicum* different media exposed to either incubator or laboratory

**Means in columns compares diameter of *E. turcicum* in a paticular media as relates to condition and time of exposure.



Graph 1- The main effects of PDA, V8-juice agar, and WA on colony diameter of *E. turcicum* averaged across the incubation conditions after 168 hours



Graph 2- The main effects of incubating *E. turcicum* under laboratory and incubator conditions

4.5.0 Survival of SLB pathogen on sorghum stubble left on the soil surface (0 cm) and buried 15 and 30 cm deep for five weeks

The survival of *E. turcicum* was significantly reduced by burial depth of infested sorghum stubble during a 5 week period. The fungus survival was 75%, 63% and 32% in stubble left on the soil surface and buried 15 cm and 60 cm in the soil, respectively (**Table 11**).

Table 11 - Survival of *E. turcicum* on stubble at three different placement levels after 5 weeks

Fungicide	*Mean growth (mm)
Impact	1.31± 0.42a
Artea	0.00± 0.00b
Propicorn	0.00± 0.00b
Average mean	0.43± 0.24
LSD (0.05)	0.85

4.6.0 Evaluating the response of thirteen sorghum varieties to E. turcicum infection under greenhouse conditions.

All the varieties evaluated in the greenhouse for their response to infection by *E. turcicum* exhibited some levels of susceptibility to the pathogen (**Table 12**). Varieties NUS421 and 510 had the highest disease severity while varieties Sephala, Segaolane, Pannar8816 and Mr Taurus had the least level of disease severity (**Table 12**).

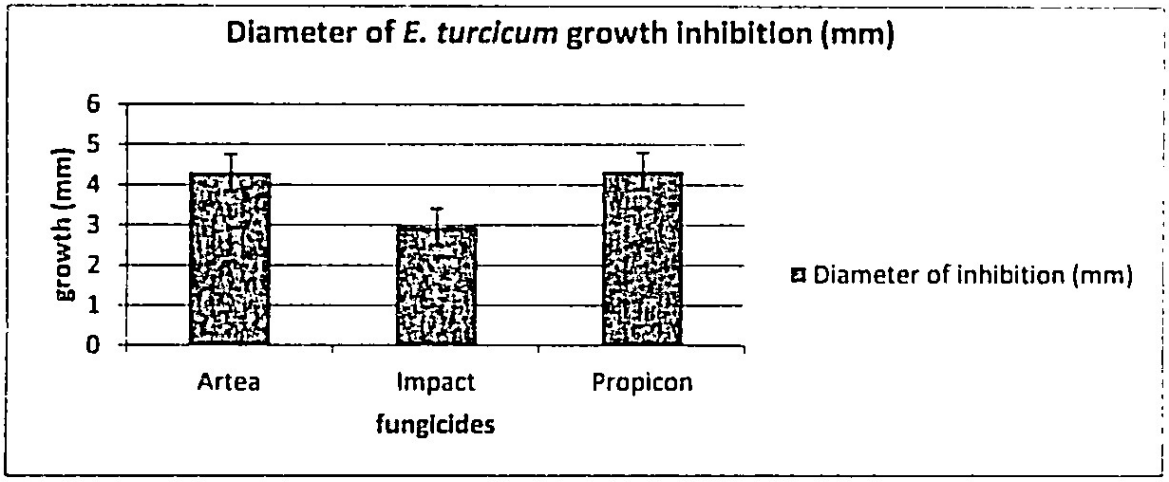
Table 12 - Response of thirteen varieties inoculated with *E. turcicum* spores14 days after inoculation in the green house

Variety	Disease severity*1	Number of plants infected*2	Infected leaves per plant*3
NUS456	2.50± 0.29d	2.80± 1.11a	4.20± 1.46a
NUS421	3.50± 0.29a	2.00± 0.84abc	3.40± 1.08ab
MR-Taurus	2.00± 0.00e	1.20 ± 0.20c	3.00± 1.48abc
NUS510	3.50± 0.29a	2.40± 0.40abc	2.00± 0.45abc
BSH 1	2.75± 0.25cd	0.80± 0.38c	1.80± 0.73abc
PANNAR8106	2.75± 0.25cd	1.80± 0.37abc	1.60± 1.03bc
MR-Buster	2.75± 0.25cd	1.00± 0.00bc	1.60± 0.93bc
NS5655	3.25± 0.25ab	1.40± 0.25abc	1.40± 0.87bc
PANNAR8816	2.00± 0.00e	1.20± 0.49bc	1.20± 0.58bc
PANNAR8909	3.00± 0.00bc	1.00± 0.45bc	1.20± 0.49bc
Sephala	2.00± 0.00e	1.40± 0.40abc	1.20± 0.58bc
Segaolane	2.00± 0.00e	0.60± 0.24c	0.80± 0.37c
Tiger	3.00± 0.00bc	0.80± 0.58c	0.60± 0.40c
Average	2.69	1.42	1.8
LSD (0.05)	0.46	1.44	2.45

*1 Severity rating: 1-5 *2 Means in each column with the same letters are not significantly different *3 Means in each column with the same letters are not significantly different

4.7.0 In-vitro response of E. turcicum to the fungicides Impact, Propicon 250 EC and Artea used by Panda farmers for the control of SLB.

Artea and Propicon were able to completely stop the mycelial growth of *E. turcicum* after their introduction to an actively growing colony (**Appendix 2**). The greater fungicide inhibition was represented by the larger diameter of clearance of the growing *E. turcicum* as compared to the less effective (**Graph 3**).



Graph 3- Diameter of inhibition of the pathogen with three fungicides (Artea, Impact and Propicon) in V8-juice agar after five days

- Diameter of inhibition of *E. turcicum* with three fungicides (Artea, Impact and Propicon) in V8-juice agar after five days

In experiment where media was mixed with the chemical, it was found that in the fungus grew on fungicide Impact but there was completely no growth of the fungus on Artea and Propicon (**Table 13**).

Table 13 - Average growth of *E. turcicum* in a pre-mix of media and three fungicides (artea, propicon and impact) after 5 days

Fungicide	***Mean growth (mm)**
Impact	1.31± 0.42a
Artea	0.00± 0.00b
Propicorn	0.00± 0.00b
Average mean	0.43± 0.24
LSD (0.05)	0.85

*means with the same letters are not significantly different p = 0.05 (LSD test)

CHAPTER 5.0 Discussion

5.1.0 Agronomy and farming system at Pandamatenga farms and Meteorological data

Monthly rainfall received during the season under study from October 2012 to April 2013 was a cumulative total rainfall of 335.5 mm for the whole season (**Appendix 3**). The season was also characterised by relatively humid conditions with moisture ranges of 49% in October 2012 to a high of 86% in January and February 2013 by Meteorological services. This provided a conducive environment for disease build up, as evidenced by the prevalence and severity of *E. turcicum* during the period under review.

All commercial farmers involved in the survey in Pandamatenga are producing sorghum under high input cropping systems. Management of the fields varied from well maintained (weeding on time, fungicides applications, crop rotation) to low maintenance where only one of the operations mentioned is done, each farmer was found to be practising at least one or more of the practices (**Table 5**). It was evident that well maintained farms had fewer visual signs of diseases, pests and weeds. All the farmers who were involved in this study identified poor drainage, *Quelea* birds and sorghum leaf blight in order of importance as their main problems. Farmers were able to differentiate leaf blight symptoms from those of leaf scorching due to stagnant water that result from poor drainage.

5.2.0 Identification and Assessment of incidence and severity of sorghum leaf blight in Pandamatenga farms for the growing season 2012/13

Exserohilum turcicum was morphologically identified as the pathogen isolated from infected sorghum plants in Pandamatenga farming area. When isolated directly from the leaves, conidiophores emerged in groups of two to ten on the leaf surface and were about 135-300µm long and 7- 11µm wide. They were flexuous, erect, with a swollen base and geniculate apex and bore typically straight and spindle-shaped conidia and sometimes

produced conidia in loose spikes. Conidia grew at the tips of the conidiophores and the conidiophore continued to grow at the apex, pushing aside the first-formed conidium (**Appendices 4 and 5**) (Fredicksen 1986). Conidia were 3-11 distoseptate, 50-144 x 18-33µm, pale to brown, wide in the middle, fuse-like to slightly curved, and tapered towards both ends (**Figure 2**). Conidia had a truncate and protuberant hilum in their basal cell which was visible as a small, thin stalk at the point of attachment to the conidiophore. The features above were identical to the descriptions of *E turcicum* by ICRISAT (1993) and Frederiksen (1986).Leaf blight has been reported as an important leaf disease in sorghum both in southern, eastern and western African countries where sorghum is grown (de Milliano, 1992; Hulluka and Esele, 1992; Thomas, 1992). Turcicum leaf blight caused by *E. turcicum* (Pass) K.J Leonard and E.G Suggs, Teleomorph: *Setosphaeria turcica* (Luttrell) Leonard and Suggs is an old disease of sorghum and maize (Agrios 2005; Ramathani *et al*, 2011). The fungus is distributed worldwide but predominant in subtropical to temperate climates. In severe cases leaf blight diminishes the photosynthetic area of the sorghum plant and result in few seeds per a panicle hence low yield, up to 100% losses were reported in other areas. There are also quarantine restrictions for *E. turcicum* in some countries (Navi *et al*, 1999). Recent studies in Uganda showed that cross infection of sorghum and maize was possible, with races 1, 2, 3 and 0 identified from sorghum-isolated *E. turcicum* (Ramathani *et al*, 2011).

All the fields that were sampled in Pandamatenga were found with varying levels of leaf blight incidence ranging from as low as 2.5% to a high of 100% in some fields. The incidence of SLB in fields in the northern plains was on average 63.8% while in fields in the southern plains was 28% (**Table 6**). This could be because the soils in the northern plains have poor drainage, thus increasing humidity and a conducive micro-climate for disease development, compared to the well-drained sandy soils and virgin soils in the southern plains with less humidity due to reduced surface moisture. Farms with the least incidence were found to have

used fungicides, weeding and /or crop rotation. Farms such as Q9, 12 and 14 were found to have ratoon sorghum plants and also at the periphery of farms Q14 and 17 although the latter was well taken care of by weeding and application of fungicides and regular inspections for initial onset of symptoms. The incidence in farms Q14 and 17 justifies the importance of removing debris or ploughing in stubble, which might act as reservoir for subsequent infestations. Sorghum leaf blight incidence among the various farms was found to be highest at 100% in farms Q9, 12 and 14 and 98% in Q1 and 17 all in the northern plains (**Table 8**). The lowest incidence was in Farms Q109 in the southern plains (6%) and Q26 in the northern plains (4%). The southern plains are characteristic loam clay soil which normally does not hold stagnant water for too long, this reduces the atmospheric moisture that favours disease development. Disease severity was highest (4.40 rating) in farm Q9 which had ratoon crops growing alongside the crop which could be the carrier of the pathogen. The lowest was recorded in farm Q28, which is relatively clean, fungicides are also used and 38 in the southern plains with less favourable conditions for disease development (1.2 rating) (**Table 7**). All the farms surveyed were planted imported sorghum hybrids MR-Buster and Pannar (PAN), and the majority (77%) of those farms were on the northern plains. That implies that most of the sorghum was planted in the black cotton soils of the northern plains than the southern plains. This could be that these imported varieties have tolerance to water logging and their early maturity enables them to be planted late when water logging is a problem at the beginning of the season.

Pandamatenga farmers are also practising conservation tillage which could also contribute towards disease development. Bowden (2000) reported that the effect of tillage on subsequent disease severity depends heavily on the previous crop. The fewer fields that recorded the least incidence had the best crop husbandry practices such as rotations with sunflower

(*Helianthus annus*) and cowpeas (*Vigna anguiculata*), using fungicides, weeding the fields during growth and winter ploughing.

5.3.0 Comparison of Water agar (WA), V8-juice agar, Potato dextrose agar (PDA) as media for growth and inoculum production of the pathogen causing sorghum leaf blight under laboratory and incubator conditions

Fungi are said to grow in different habitats in nature and are cosmopolitan in distribution requiring several specific elements for growth and reproduction (Sharma and Pandey, 2010). In the laboratory fungi are isolated on specific culture medium for cultivation, preservation, macroscopic examination and physiological characterisation. A wide range of media are used for isolation of different groups of fungi that influence the vegetative growth and colony morphology, pigmentation and sporulation depending upon the composition of specific culture media, pH, temperature, light, water availability and surrounding atmospheric gas mixture (Sharma and Pandey, 2010). Physical and chemical factors have a pronounced effect on the diagnostic features of a fungus. It is therefore necessary to use several media while attempting to identify a fungus in culture since mycelial growth and sporulation on media are important components of identification.

Production of conidia was supported by all three media PDA, WA and V8-juice agar. Mycelial growth on V8-juice agar was found to be always better than PDA and WA. After 24 hours the pathogen in V8-juice agar had shown its potential of active growth and its colony diameter was the highest at the end of the experiment (**Figure 4**). V8-juice agar had the highest growth at 48 hours of 8.88 mm and the least was WA at 3.38 mm wide, and at the end of the experiment V8-juice agar was still the highest at 19.14 mm with WA still least at 8.53mm after 168 hours (**Table 10**). This suggests that the pathogen will produce more mycelium within a short period of time onV8-juice agar and produce spores within 24 hours

on WA making the two media useful for immediate identification of the pathogen. PDA was generally a poor medium for mycelial growth and sporulation of *E. turcicum*.

There was generally no difference between *E. turcicum* when the culture was allowed to grow in the incubator at controlled temperature and light regimes or at room temperature. In both treatments colony diameter after 168 hours averaged 13.79 mm and 11.87 mm in the incubator and the lab respectively (**Graph 2**). Although general growth was not affected by change of temperatures, there was a variation on individual medium performance with V8-juice having the highest mean growth of 16.00 mm in the laboratory and 22.23 mm in the incubator. The SLB pathogen responded differently in PDA, WA, and V8-juice agar (**Graph 1**). The pathogen appeared velvety in texture for all the media, with generally smooth margins and a greyish reverse colour. It also produced conidia in all the treatments at different times 24 hours in WA, 48 hours V8-juice agar and only after 7 days in PDA.

Sporulation was more severe in WA (**Table 9**) than the two culture media as the clusters of spores were found to be larger in WA than the other two media.

The results are in line with the findings of Chang and Fan, (1986) that isolates of *E. turcicum* performs fairly stable in V8-juice agar in maintaining original vigour after few generations on laboratory media. The results also showed that cultures derived from cultures previously grown on 1% WA always grew normally with a thin and smooth colony than on other laboratory media such as PDA and V8-juice agar.

5.4.0 Survival of the pathogen on sorghum stubble left on the soil surface (0 cm) and buried 15 and 30 cm deep.

The effect of tillage on subsequent disease severity depends heavily on the previous crop (Bowden, 2000). For example, crop debris from sorghum contains numerous pathogens which can harm a subsequent sorghum crop. Therefore, direct drilling sorghum under no-

tilling system amongst sorghum stubble from previous season often results in high disease severity. On the other hand, crop debris from legumes contains very few pathogens that can harm sorghum. Therefore, no-tilling sorghum into legume stubble poses little risk. Local epidemics of northern leaf blight, incited by the fungus *Exserohilum turcicum* on maize are found to usually originate from conidia on infested maize residues (Adipala and Ogenga-Latigo, 1994).

Exserohilum turcicum of sorghum also survives sorghum residues from the previous infection. Survival of *E. turcicum* was highest (75%) on the surface and lowest (32%) in infested residues buried 30 cm in the soil. The results show that conventional tillage or residue burial is an important measure of suppressing the pathogen survival on sorghum residue and should be a component of integrated disease management of sorghum turcicum leaf blight.

5.5.0 Response of thirteen sorghum varieties to E. turcicum infection under greenhouse conditions

Sorghum (*Sorghum bicolor*) is frequently devastated by turcicum leaf blight caused by *E. turcicum* leading to considerable grain and fodder losses. Geenrally all varieties that were tested responded to *E. turcicum* infection. Different sorghum varieties have different levels of infection by *E. turcicum*. The varieties did not have a significant difference in the number of plants infected although a maximum mean of 3 plants infected was found in NUS456 and a minimum mean of 1 infected plants of segaolane. The number of leaves infected per plant was not significantly different, but ranged from 1 in variety Tiger to 4 in NUS456 after cover spraying individual plants with *E. turcicum* spores.

The variation could also be due to differences in defence mechanisms depending on the breed of sorghum. There was a significant difference in level of leaf necrosis for all the varieties

infected with *E. turcicum*, the highest rating being 3.50 for varieties NUS421 and NUS510 and the least of 2.00 for MR-Taurus, PANNAR 8816, sephala and segaolane, respectively (Table 12).

5.6.0 In-vitro response of E. turcicum to the fungicides Impact, Propicon 250 EC and Artea used by Panda farmers for the control of SLB.

Plant diseases caused by fungi cause significant damage and economic losses in crops every year (Lucas, 2001). It is difficult however to predict whether a disease is likely to occur in a particular crop and how severe it will become. As a consequence farmers in Pandamatenga purchase Artea, Propicon and Impact for any possible occurrence of *E. turcicum* and use them as a rotational spray. The application of fungicides is an important way of increasing or maintaining good yields in cereal crops. In a study by the South African Barley Breeding Institute (SABBI) it was found that triazole application on barley against net blotch gave very good results, with relatively low costs per hectare. However the resultant effect of different fungicides and different application doses depends on the weather, soil and management conditions. Currently three different fungicide grouped Demethylation- Inhibitor (DMIs), the trizoles and the strobilurins) (www.sabbi.org, 2009; Tortora et al, 2002) are mainly used for fungal control in cereals. DMIs (i.e. Artea, Impact, Propicon) are also known as sterol biosynthesis inhibitors or ergosterol biosynthesis inhibitors. They show varying degrees of systemic activity. There is potential for cross-resistance amongst all the active ingredients within the DMI fungicides (www.sabbi.org, 2009).

Propicon and Artea were found to be effective in inhibiting *E. turcicum* in-vitro. Propicon and Artea suppressed mycelial growth and caused the fungus not to grow beyond the point of their placement proving to be effective to control *E. turcicum* when a disc with fungicide was placed on an actively growing colony but Impact continued to support growth of the pathogen

(**Appendix 2**). Artea and propicon premixes with media did not allow any new growth of *E. turcicum* unlike Impact where a new colony developed (**Table 13**). Artea and propicon are therefore recommended for management of sorghum turcicum leaf blight and Impact is not recommended. Either one of them could be included in a rotational spray with any other product outside the triozole group; this will reduce chances of the development of fungicidal resistance. The three fungicides are in the same group of triazoles, therefore they are not supposed to be used as rotational sprays on their own. This might only predispose the pathogen to develop fungicide resistance and also unnecessary costs of production to the farmer. The three fungicides could therefore be used interchangeably with strobilurins and many other registered products according to the Agrochemical's Act, (2007).

CHAPTER 6.0 Conclusion

The pathogen causing SLB in Pandamatenga farms was identified as *Exserohilum turcicum*. Sorghum leaf blight is well spread in Pandamatenga and all the farms that were studied had some level of turcicum leaf blight infections. It is relatively easy to get the fungal cultures under both laboratory conditions and controlled environment (incubation) with various media that are available such as V8-juice agar, Potato dextrose agar and Water agar. V8-juice agar is the best medium for mycelial growth and WA is good for sporulation of the pathogen. *E. turcicum* can survive on sorghum stubble/ crop residues left on soil surface. Survival of *E. turcicum* over time is drastically reduced by depth of stubble in the soil, the deeper the crop residue in the soil the lower the amount of initial inoculum of the pathogen. Different varieties of sorghum grown in Pandamatenga including MR-buster which were subjected to *E. turcicum* leaf blight spores reflected the initial symptoms identified for *E. turcicum*. Chemical control is a requirement especially when all preventative measures have proven futile. Only agrochemicals that are recommended for control of *E. turcicum* Artea and Propicon had some fungistatic response to the pathogen.

CHAPTER 7.0 Limitations of the study and Recommendations

7.1.0 Limitations of the study

The study was limited by lack of equipment to use for certain experiments e.g. haemocytometer for counting spores. Funding was not available to accurately identify the pathogen using molecular techniques. Also the available facilities were so limited such that the experiment was carried out in different laboratories. The limitation also resulted in surveillance in the Pandamatenga being carried out once.

7.2.0 Recommendations for further studies

1. Molecular identification *E. turcicum* to determine the strain found in Pandamatenga commercial farms,
2. Distribution of *E. turcicum* among the sorghum producing areas in Botswana and its impact on the yield in both commercial and subsistence setups,
3. Determination of micro-climatic conditions favourable for the infestation and spread of *E. turcicum* in a sorghum crop,
4. Evaluation of all local landraces on resistance to *E. turcicum* and the genes coding for resistance and establishing the gene coding for resistance in sorghum,
5. Determination of alternative wild hosts of *E. turcicum* in Botswana.
6. Evaluation of more fungicides with different modes of action for use in the control of *E. turcicum*
7. Integrated disease management programme for *E. turcicum* in Pandamatenga

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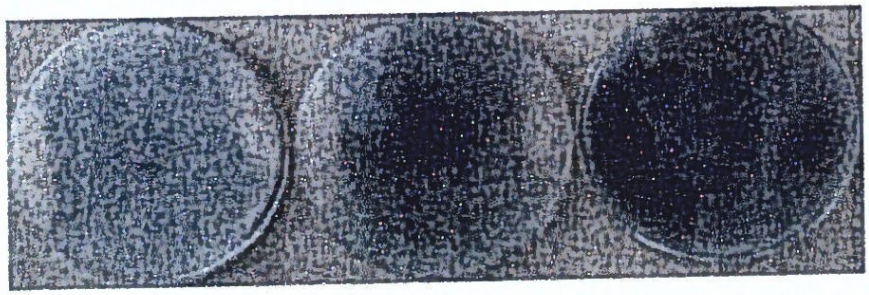
CHAPTER 9.0 APPENDICES

Appendix 1: ANOVA table for Media testing experiment

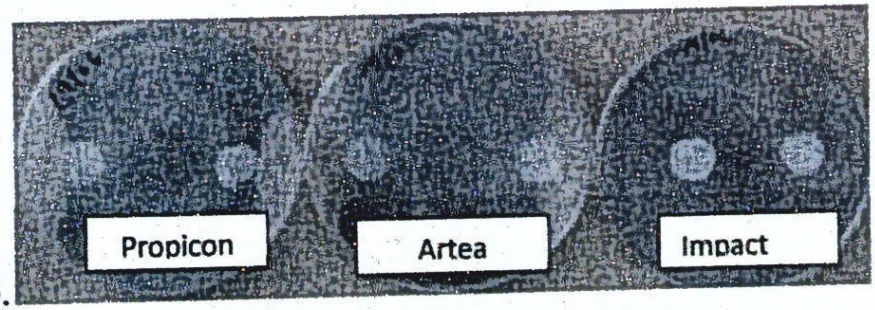
Effect	Num DF	Den DF	F Value	Pr > F
Place	1	3.89	16.33	0.0165
Treat	2	61.3	510.94	<.0001
Cond*Treat	2	61.3	71.44	<.0001
Time	4	29.4	937.33	<.0001
Cond*Time	4	29.4	11.11	<.0001
Treat*Time	8	29.4	53.64	<.0001
Cond*Treat*Time	8	29.4	6.83	<.0001

Appendix 2: Growth patterns of *E. turcicum* with fungicides filter discs after 4 days of exposure **a.** back view **b.** view from top

a.



b.

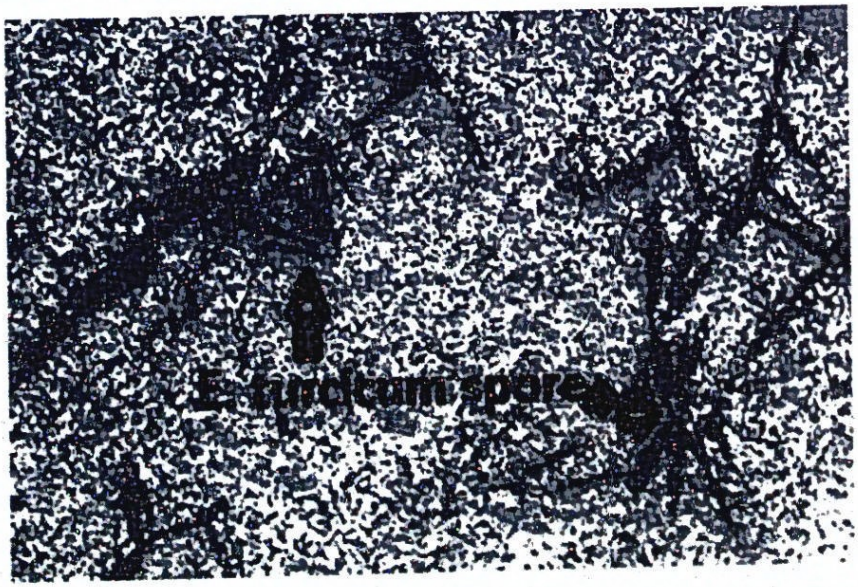


Appendix 3: Monthly total rainfall (mm) from October 2012 to June 2013 in Pandamatenga

Month	Rainfall (mm)	Relative Humidity %
October	2.8	49
November	60.9	55
December	55.5	79
January	211.5	86
February	38.2	86
March	3	78
April	1.8	68
May	0	63
June	0	69
Total	**335.5**	

Source: Department of Meteorological Services, Gaborone

Appendix 4: *E. turcicum* spores in Water Agar after 168hrs incubation at room temperature

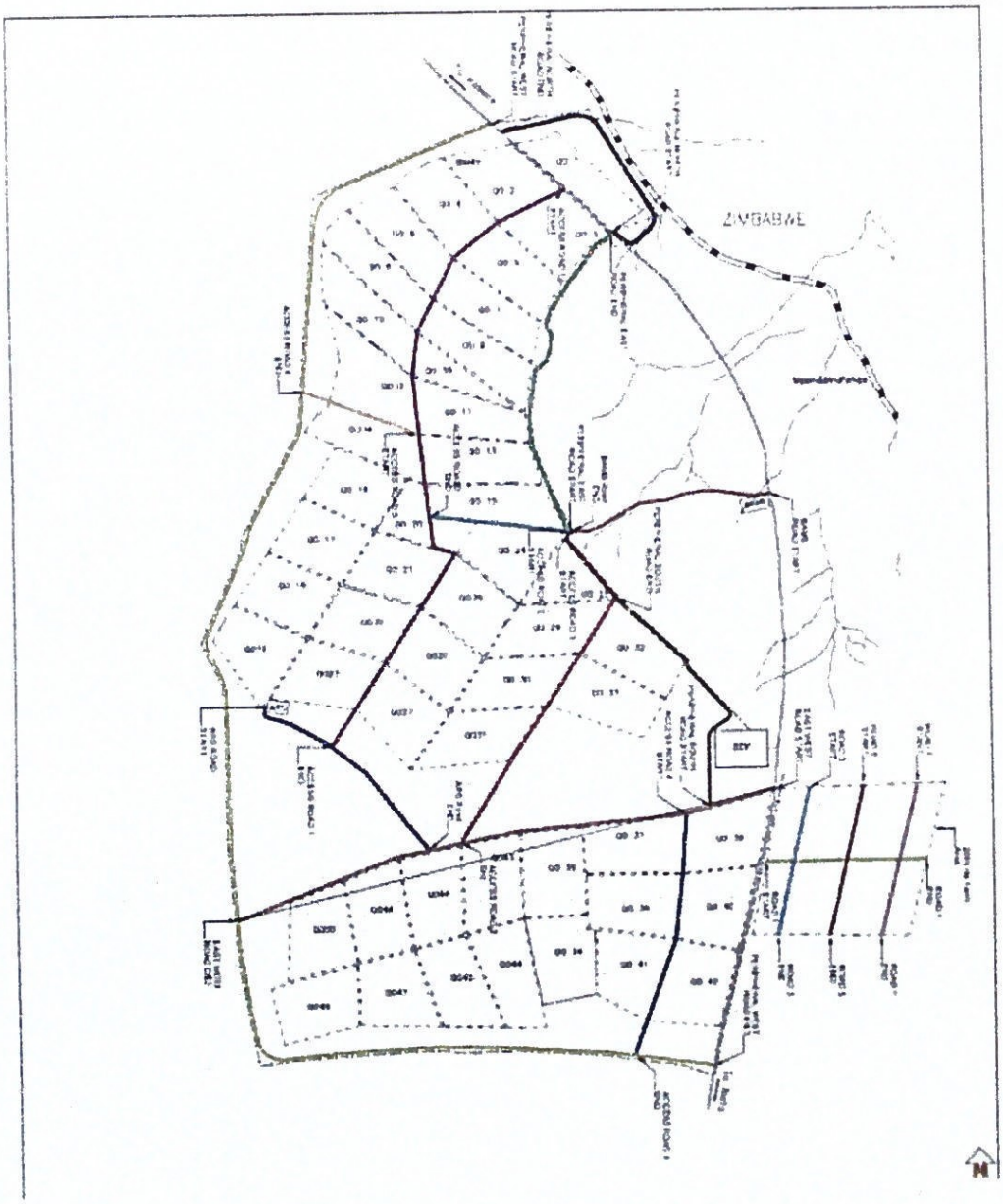


Appendix 5: *E. turcicum* sporulation in an old leaf disk from a moist chamber after 48 hours of incubation

Appendix 6: MR-Buster with very severe leaf blight symptoms (level 4.40) and 100% incidence at farm Q9 during the ploughing season 2012/13

Appendix 7: Infected leaves showing varying degrees of leaf necrosis left-right, **a.** free from disease, **b.** Slight symptoms, **c.** moderate symptoms, **d.** Moderately severe symptoms, **e.**very severe

Appendix 8: Map of Pandamatenga farms



Appendix 9: List of pathogens controlled by the triazole fungicides Artea, Impact and Propicon commonly used by farmers in Panadamatenga

Pathogen	Disease	Artea	Impact	Propicon
Rhynochosporium secalis	Leafspot	✓	✓	✓
Pyrenospora spp	Netblotch	✓	✓	
Helminthosporium spp	Leaf blotch		✓	
Erysiphe graminis	Powdery mildew	✓	✓	✓
Puccinia spp	Leaf rust	✓	✓	✓
Septoria spp	Leaf/glume blotch	✓	✓	✓
Exserohilum turcicum	Leaf blight	✓	✓	

Source: Enclosed pesticides manufacturers labels