The effect of plant extracts on anthracnose of *Phaseolus vulgaris* L. and *Vigna unguiculata* (L.) Walp

by

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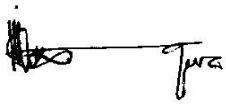
DECLARATION

I, Johnny Isaac Gregorio Masangwa, declare that the thesis, which I hereby submit for the degree Master of Science at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

Signature: 

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SUMMARY

Anthracnose is one of the serious diseases of cowpea (*Vigna unguiculata* L. Walp) and common bean (*Phaseolus vulgaris* L.) caused by the *Colletotrichum* fungi. The disease is prevalent is small holder farmers' fields due to the scarcity and high cost of the synthetic fungicides. This study was conducted with the main aim of improving food security and income of the smallholder farmers by increasing legumes, *P. vulgaris and V. unguiculata* thereby increasing production and improve food security and income of smallholder farmers. Investigations involved *in vitro* bioassaying for antifungal activities of the crude extracts on *Colletotrichum lindemuthianum* (Sacc. and Magn.) Bri. and Cav. and *Colletotrichum dematium* (Fr.) Grove var. *truncata* field isolates and evaluating the effect of crude plant extracts seed treatments on seed germination, emergence and control of anthracnose disease of common bean and cowpea. Furthermore, ultra-structural changes of plant extracts treated and efficacy of foliar application of extracts. The *in vitro* study showed that *Allium sativum* L., *Agapanthus caulescens* Spreng., *Carica papaya* L. and *Syzygium cordatum* Hochst.ex Krauss extracts have good antifungal activities against both *C. lindemuthianum* and *C. dematium.* The low concentrations (5 $mg.ml^{-1}$) of *Syzygium* and *Agapanthus* water extracts and acetone extracts of *Agapanthus* and *Carica* gave a high percentage of bean seed germination, emergence, short mean emergence time (MET) and were effective in controlling the anthracnose disease. The treatment of *Agapanthus* (both water and acetone) extracts also increased the shoot length and dry weight of the seedlings. The *Allium* acetone extracts (5 $mg.ml^{-1}$) was the only treatment that gave good results with respect to germination percentages, MET, shoot length, leaf area and dry mass of cowpea. Five $mg.ml^{-1}$ concentrations of *Syzygium* and *Agapanthus* water extracts and acetone extracts of *Agapanthus* and *Carica* have potential as seed treatments on bean. *Allium* acetone extract (5

mg.ml^{-1}) was the only potential cowpea seed treatment that could be recommended to farmers as an alternative to the synthetic fungicide. Electron microscopy revealed that principle differences were observed in the cotyledon-embryo connecting tissues of seeds treated with *Agapanthus*, which had few cristae in their mitochondria than the cells from other treatments. The embryonic root cells of bean seeds treated with *Agapanthus* had coalescing protein bodies. The embryonic root cells of cowpea and bean treated with *Syzygium* had fewer lipid bodies as compared to the control and the *Agapanthus* treated seeds. Bean plants that were foliar treated with the 15 mg.ml^{-1} concentrations of *Allium* water, *Agapanthus* water, *Carica* water, *Agapanthus* acetone, *Carica* 5 and 15 mg.ml^{-1} acetone, *Syzygium* 5 mg.ml^{-1} acetone extracts and the combinations (2.5 mg.ml^{-1} + 2.5 mg.ml^{-1}) of *Allium* + *Agapanthus*, *Allium* + *Carica*, *Agapanthus* + *Syzygium* and *Carica* + *Syzygium* extracts registered low anthracnose (*C. lindemuthianum*) disease severity and high leaf area. The cowpea plants treated with 15 mg.ml^{-1} water extracts of *Agapanthus* and the combinations of *Allium* + *Agapanthus*, *Agapanthus* + *Carica* and *Agapanthus* + *Syzygium* extracts recorded low cowpea anthracnose (*C. dematium*) disease severity, highest leaf area and dry mass. The study revealed that *A. sativum*, *Agapanthus*, *C. papaya* and *S. cordatum* plant extracts have antifungal activities and can be used as alternative seed treatments and foliar fungicides against the anthracnose diseases of legumes (cowpea and common bean) instead of synthetic fungicides without causing any negative effect on seed germination, emergence, ultra-structure of seeds and plant growth.

DEDICATION

I dedicate this thesis to my late dad, ***Mr Isaac Gregorio Johnny Masangwa,*** a man who strived hard so that I could excel in life and attain a higher level of education in spite of his meagre financial resources. He said bye to this cruel world when I was busy sitting for my BSc final exams at University of Malawi before seeing the fruits of his hard work of encouraging me to work hard in class. "*May your soul rest in peace!!!!* "

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CHAPTER ONE

GENERAL INTRODUCTION

1.1 BACKGROUND

Common bean (*Phaseolus vulgaris* L.) and cowpea (*Vigna unguiculata* L. Walp) are important legume crops in the Southern Africa Development Community (SADC) region. These two crops are grown for food, cash, animal feed and as soil improvers. Cowpea is also reported to possess medicinal value (van Wyk and Gericke, 2000; Kritzinger, 2005). Common bean and cowpea seeds have metabolites which have antifungal and antibacterial activity against some plant pathogens (Ye *et al.*, 2000; Ng, 2004). These chemicals also have the ability to inhibit cancer and reduce HIV-1 reverse transcriptase activity (Ye *et al.*, 2000; Ng, 2004; Wong and Ng, 2005; Wong *et al*., 2006; Lam and Ng, 2009). Farmers who grow these crops do not realize potential yield due to several limiting factors amongst which are the incidence and severity of diseases (Coyne *et al.,* 2003). Anthracnose is one of major fungal diseases caused by *Colletotrichum lindemuthianum* (Sacc. and Magn.) Bri. and Cav. in common bean and *Colletotrichum dematium* (Fr.) Grove var. *truncata* in cowpea in the SADC region (Adebitan *et al*., 1992; Allen, 1983; Allen *et al.*, 1996; Emechebe and Florini, 1997). Bokosi (1986) and Diaz and Lopez (1986) reported that severe infection of this disease on common bean may result in total loss of the crop. Emechebe and Florini (1997) reported that 50 % cowpea yield loss can occur in susceptible varieties. This disease may appear in the field about 4-6 weeks after seedling emergence. Symptoms may appear on leaves, stems and pods. The most striking symptoms appear on pods. Later spots develop large, dark, more or less circular spots with sunken centers and raised margins (Bokosi, 1986).

There are major ways of controlling anthracnose disease such as use of resistant varieties, disease free seed and use of synthetic fungicides. Scientists are continuing developing bean varieties that are resistant to anthracnose disease (Beaver *et al.*, 2003). However, problems are encountered due to variability in terms of a number of old and emerging new anthracnose pathogen races (Balardin *et al.*, 1997; Bigirimana and Hofte, 2001). Use of diseased free seed is another aspect that is difficult to regulate especially in Africa where farmers like to exchange seed (Dron and Bailey, 1995; Nkongolo 2003; Kapila, 2008). This seed exchange behaviour also contributes to the distribution of seed-borne diseases especially that of anthracnose as the pathogen is seed-borne (Dron and Bailey, 1995).

The only effective way of controlling the disease is by the use of synthetic fungicides, however, there are several demerits that have been reported against their use (Falandysz, 2000; Voorrips *et al.*, 2004; Shovan *et al.*, 2008, Edwards, 2010). Synthetic chemicals often lead to increased resistance to diseases, weeds and insect pests alike, as well as the appearance of other diseases formerly unknown (Avenot and Michailides, 2007; Anonymous, 2011; Davis, 2011). Accumulation of harmful chemical residues in grain, water and soil has also been reported after continuous use of synthetic chemicals (Falandysz, 2000; Voorrips *et al.*, 2004; Shovan *et al.,* 2008). Wilson *et al*. (1997) reported that fungicide food residues pose more carcinogenic risks than the insecticide and herbicide residues.

Several plants possess insecticidal, fungicidal, bactericidal, as well as viricidal properties in their leaves, seeds, stems and even roots. According to Maulana (2000), botanical fungicides and bactericides (often referred to as simply “botanicals”) are products that are derived from natural plant compounds used to control diseases in various ways. Botanicals are reported to be superior to synthetic fungicides because these natural compounds are usually biodegradable, environmentally safer and they are easier, and cheaper

to obtain than the synthetic insecticides (Amadioha, 2000; Maulana, 2000; Tripathi and Dubey, 2004). Once applied, botanicals leave no or little harmful residues (Edwards, 2010). There has been a shift from synthetic chemicals to natural compounds because of the merits mentioned above and several researchers are testing traditional medicinal plants for the control of plant diseases (Islam *et al*., 2001; Nwachukwu and Umechuruba, 2001; Alabi *et al*., 2005; Raghavendra *et al.,* 2006; Shafique *et al.,* 2007; Somda *et al.*,2007; Akinbode and Ikotun, 2008).

1.2 JUSTIFICATION OF THE STUDY

Currently the only effective way of reducing anthracnose disease incidence and severity is by the use of synthetic chemical fungicides. In most African countries, smallholder farmers fail to apply synthetic chemical fungicides to their crops because they are too expensive or not available (Somda *et al.,* 2007). Smallholder farmers need to be given cheap technologies that are safe, ecologically sound and at the same time very effective in controlling *Colletotrichum* spp. and that are similar to synthetic chemical fungicides that reduce disease losses and in turn realize increased production per unit area. Studies suggest that there are many substances naturally occurring in plants that are toxic to *Colletotrichum* fungi (Amadioha and Obi, 1998; Charigkapakrn, 2000; Alam *et al.,* 2002; Bautista-Banos *et al.,* 2003; Raghavendra *et al.,* 2006; Chowdhury *et al.*, 2007; Nduaga *et al.,* 2008; Shovan *et al.,* 2008). Much research on botanicals has been done on the anthracnose diseases of vegetables (Kraikruan *et al*., 2008; Nduagu *et al.*, 2008) and fruits (Alam *et al*., 2002; Bautista-Baños, 2003; Chowdhury *et al.*, 2007) and other diseases caused by soil-borne pathogens such as *Pythium*, *Rhizoctonia* and *Fusarium* (Pretorius *et al.*, 2002; Minuto *et al.,* 2006; Tegegne *et al*., 2008) but little research and development has been done on *Colletotrichum* of cowpea and common bean and it was against this background that the

research project was conducted.

1.3 FUNDAMENTAL OBJECTIVE

The research project was conducted with the main objective of improving food security and income of the smallholder farmers by increasing legume (*Phaseolus vulgaris* and *Vigna unguiculata)* production, reducing the disease incidence and severity through the use of effective botanicals as an alternative to synthetic fungicides.

1.4 SPECIFIC OBJECTIVES

Specifically the project aimed at:

1. Screening the crude plant extracts that can be used in controlling anthracnose disease of legumes.
2. Evaluating the effect of crude plant extracts as a seed treatment on cowpea and bean seed germination, emergence and control of anthracnose disease.
3. Studying the ultra-structural changes that occur within the seed cells after crude plant extracts treatment.
4. Evaluating the efficacy of foliar application of crude plant extracts on the anthracnose diseases.
5. Evaluating the efficacy of the combination of different crude extracts in the control of anthracnose disease development.

1.5 HYPOTHESES TESTED

The hypotheses that were tested in this study were:

a. There is no inhibition of growth of *Colletotrichum* fungi *in vitro* by different crude extracts.

b. There are no differences among different plant extracts in their effectiveness in reducing the anthracnose disease incidence and severity.
c. There is no effect of crude plant extracts on seed germination and emergence.
d. There are no ultra-structural differences in the crude plant extract treated and untreated seeds.
e. There are no differences in the effectiveness between the combined crude plant extracts and a single crude plant extract in controlling anthracnose disease.

1.6 CHAPTER OUTLINE

Chapter 2. The literature review of the crops (cowpea and common bean), *Colletotrichum* species (anthracnose pathogens) that affect cowpea and common bean and botanicals (natural plants), which were used in the research project, is given.

Chapter 3. Acetone, ethyl acetate and water extracts from six different plants were tested *in vitro* for their fungicidal properties against the two *Colletotrichum* species (*C. dematium* and *C. lindemuthianum*). The inhibition properties of the extracts are discussed here.

Chapter 4. The effect of acetone and water extracts from four selected plants (*Agapanthus caulescens* Spreng., *Carica papaya* L., *Allium sativum* L. and *Syzygium cordatum* Hochst.ex Krauss) on cowpea and bean seed germination, viability, emergence and control of seed-borne anthracnose disease were evaluated. The effects of these extracts on the four mentioned parameters are thoroughly elucidated here.

Chapter 5. The ultra-structural changes of cowpea and bean seed treated with plant extracts by soaking were studied and are discussed in this chapter.

Chapter 6. The efficacy of water and acetone plant extracts from the four selected plants (as used in chapter 4) and the synergies of the combination of water extracts in controlling anthracnose disease of beans and cowpea *in vivo* were evaluated and discussed.

Chapter 7. This chapter includes the general discussion, interpretation of results and comments pertaining to the experiments conducted. Short-comings of the study as well as recommendations are made.

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CHAPTER TWO

LITERATURE REVIEW

2.1 LITERATURE REVIEW OF THE CROPS

2.1.1 Common bean (*Phaseolus vulgaris* L.)

2.1.1.1 Origin and production

The common bean (Figure2.1) is a member of the Fabaceae family and the tribe Phaseoleae. The crop is commonly known as harot bean, field bean, kidney bean, snap bean and French bean (Kay, 1979). The crop is also known by several local names like nyemba (Chichewa-Malawi), bwanda (Sena and Mang'anja-Malawi), diwana (Isuzulu, Sotho, Tswana - RSA), groenboon (Afrikaans - RSA). The common bean is the most important grain legume for direct human consumption in the world (CIAT, 1993). It is globally ranked number three as a food legume after soybeans [*Glycine max* (L.) Merr.] and peanuts (*Arachis hypogaea* L.) (Lin *et al.,* 2008).

The common bean is believed to have originated in Latin America. The region is the leading producer of bean and produces almost two fifths of the world's production (Kay, 1979; Pachico, 1989). Reports indicate that bean was introduced to Africa by the Portuguese and Spanish (Cobley and Steele, 1976; Baudoin *et al*., 2001) and the continent is a secondary centre for genetic diversity (CGIAR, 2011). Africa is the second leading producer of bean and accounts for two million tons, 25 % of the world's annual production from 3,741, 000 hectares put under bean cultivation (Pachico, 1989; Allen, 1995; Wortmann *et al*., 1998). In Africa, the sub-Saharan region is the largest producer of bean. Southern Africa accounts for 32 % of bean produced in Africa. In tropical Africa, the Democratic Republic of Congo, Rwanda, Burundi, Ethiopia, Kenya, Uganda, Tanzania, Malawi, Angola and Mozambique are

the leading producers (Brink and Belay, 2006). The crop is mainly produced for subsistence by smallholder farmers. Bean is grown either as a sole crop or intercropped with other crops. The sole bean crop production amounts to 42 %; maize (*Zea mays* L.) intercropped 47 %; root/tuber intercrop 6 %, millet (*Panicum miliaceum* L.) /sorghum (*Sorghum bicolor* L. Meonch) intercrop less than 1 % and 4 % is intercropped with other crops (Wortmann *et al*., 1998). Brink and Belay (2006) reported that bean is sometimes grown on residue moisture as a relay crop in Malawi and Tanzania.

2.1.1.2. Morphology of common bean

Common bean is an annual herbaceous plant that has variations in vegetative characters, flower colour, and size, shape and colour of the pods and seeds. The growth habit can be determinate, indeterminate or intermediate (Purseglove, 1968; Kay, 1979). Beans have a tap root that can grow up to 90 cm and some lateral roots that bear nodes are confined to 15 cm of the top soil. The stems are slender, twisted, angled and can be 20 – 60 cm in the dwarf type but up to 2 – 3 cm in the climbing type (Purseglove, 1968; Cobley and Steele, 1976; Kay, 1979). Beans have hairy, alternate, trifoliate leaves that have long petioles. The leaflets are ovate, entire, acuminate and measure 8-15 x 5 -10 cm long. Flowers are either white, creamy-yellow, pink or violet and are self-pollinated. The fruits (pods) are glabrous, straight, or slightly curved and measure 7.5 – 20 x 1.0 – 1.5 cm long and contain 4 - 12 seeds. The seeds vary in colour, size and shape and weight of 100 seeds is 20 – 60 g (Purseglove, 1968; Kay, 1979).

Figure2.1: Common bean plants (Masangwa, J., 2010).

2.1.2.3. Importance of common bean

The types of bean grown in southern African countries are used mostly as dry seeds, although fresh, mature green-shelled seeds and immature pods are also cooked and eaten. Green tender leaves, *khwanya* (Chichewa - Malawi), are sometimes picked before pods set and eaten as a green vegetable (Msuku *et al.*, 2000). Bean leaves are commonly picked and sold fresh. The leaves are picked from the second or third trifoliate stage through to flowering. Bean leaves are an important component of diets since they contain about the same amount of protein as dry seed on a dry weight basis (Allen, 1986). According to Jones (2011), beans are a major source of protein in some African countries like Kenya, Tanzania, Malawi, Uganda and Zambia. According to Msuku *et al*. (2000) smallholder farmers like to grow beans because they are easier to store when they are dry than beef, poultry, or fish. Kadyampakeni (2004) reported that, as food, beans provide a cheap source of protein for the majority of the people who are far from the lakeshore areas of Malawi where fish is the main source of protein as evidence is now suggesting dwindling catches. Beans are also high in calcium, magnesium and iron and contain large amounts of vitamin B (Edje *et al.*, 1980). Cooked beans can

provide 132 g/kg proteins, 33 g/kg lipids, 38 g/kg fiber, 24 g/kg ash and 755 g/kg carbohydrates (Mora-Aviles *et al.* 2007) but mature dried beans contain 11.0% water; 22.0 % protein; 1.6 % fat; 57.8 % carbohydrate; 4.0 % fibre; 3.6 % ash; 137mg/100 g calcium; 6.7 mg/100 g iron; vitamin A 30 iu/100 g; thiamine 0.54 mg/100 g; riboflavin 0.18 mg/100 g; niacin 2.1 mg/100 g and ascorbic acid 3.0 mg/100 g (Kay, 1979).

Beans are a source of income to the commercial farmers and also to some subsistence smallholder farmers who dominate its production in Africa and sell part of their production to the urban populations (Jones, 2011). Like all other legume crops, common bean symbiotically fix nitrogen in the soil but Olivera *et al.* (2004) reported that the fixation potential of bean is low as compared to other legumes. The bean nitrogen fixation is affected by availability of phosphorus (P) such that when there is high availability of P in the soils, the beans produce many nodes (Olivera *et al.*, 2004) and this in turn increases the amount of nitrogen fixed in the soil. Cardoso *et al.* (2007) reported that nodulation and biological nitrogen fixation of common bean is negatively affected by the presence of high mineral nitrogen in the soil but nodulation and nodule longevity is high in an intercropped setting than in sole cropping.

According to Brink and Belay (2006), the powder of the carbonized bean seeds is applied to wounds as a medicine in Mali. The studies by different researchers indicated that *Phaseolus* beans have chemicals that have antifungicidal, antibacterial, anticancer as well as HIV-1 reverse transcriptase activities (Ng, 2004; Wong and Ng, 2005; Wong *et al.*, 2006; Ma *et al.,* 2009; Lam and Ng, 2009). Peptide, hemagglutinin and mitogenic defensin from haricot beans, French beans and white cloud beans, respectively, are reported to be responsible for the activities mentioned above (Wong and Ng, 2005; Wong *et al.*, 2006; Lam and Ng, 2009). Peptide and hemagglutinin have also been reported to have antifungal activity against phytopathogenic fungi such as *Fusarium oxysporum* Schlechtend.:Fr., *Rhizoctonia solani*

Kühn, *Bipolaris maydis* (Nisik and Miyake) Shoemaker. and *Botrytis cinerea* Pers.:Fr. (Wong and Ng, 2005; Wong *et al.*, 2006). Wong and Ng (2005) also reported that a bean peptide called vulgarinin has an inhibiting activity on the growth of plant pathogenic bacteria like *Proteus vulgaris*, *Bacillus subtilis*, *Bacillus megaterium* and *Mycobacterium phlei*. Anti-cancer activity was reported to be due to the anti-oxidative characteristics of phytochemicals that are high in seed coats of common bean (Jeng *et al.,* 2009).

2.1.1.4 Common bean field disease constraints

Despite all the above attributes, common bean has current average yields of about 500 kg/ha, far below its potential yield, which is over 3000 kg/ha (Msuku *et al*., 2000). Farmers fail to realize high yield per unit area due to a number of factors and disease attack is one of them. Chiumia *et al.* (2003) reported that common bean is widely susceptible to diseases such as Bean Common Mosaic Virus (BCMV), Bean Yellow Mosaic Virus (BYMV), bacterial wilt, bacterial brown spot, angular leaf spot (ALS), anthracnose and rust (Allen *et al*., 1989; Msuku *et al.*, 2000). Though the crop is attacked by many fungal diseases, the most serious seed-borne fungal disease is anthracnose (Beaver *et al*., 2003). Bean anthracnose is a major fungal disease in all bean growing areas in Malawi and can cause a total loss of the crop under severe conditions like cool and moderate temperatures especially in susceptible varieties (Bokosi, 1986; Diaz and Lopez, 1986). This disease incidence occurs in the field about 4-6 weeks post seedling emergency. Symptoms may appear on leaves, stems and pods. The most striking symptoms appear on pods. Later spots develop large, dark, more or less circular spots with sunken centres and raised margins (Bokosi, 1986).

2.1.2 Cowpea (*Vigna unguiculata* L. Walp)

2.1.2.1 Origin and production of cowpea

Cowpea belongs to the family Fabaceae and tribe Phaseoleae (Padulosi and Ng, 1997). According to Purseglove (1974), cowpea is the second most important legume in Africa after common bean. Black eyed pea and southern pea are common names of cowpea. The crop is has many local names such as dinawa (Sotho, Tswana), munawa (Venda), akkerboon (Afrikaans) (van Wyk and Gericke, 2000), khobwe or nseula (Chichewa - Malawi) and nyemba (Sena and Mamg'anja-Malawi) (Nkongolo, 2003; Kapila, 2008). Cowpea culture is believed to have started long before the days of Christ in areas now called India. Cowpea was known in Asia around the year 2300 BC and in Europe to be known under the name *Phaseolas*, *Phaseolus* or *Phaselus* (Burkhill, 1953 in Pakela, 2003) but the archaeological evidence has resulted in contradicting views supporting Africa as origin (Padulosi and Ng, 1997). The centre of maximum diversity of cowpea is West Africa especially in the savannah region areas of Nigeria, southern Niger, part of Burkina Faso, northern Benin, Togo and the north western part of Cameroon (Ng and Macheral, 1985). However, the presence of the most primitive wild cowpea in southern Africa leads to the conclusion that this is the centre of genetic diversity (Tindall, 1983; Padulosi *et al*., 1990). The former Transvaal in South Africa was postulated to be the most probable centre of speciation of cowpea due to the presence of its wild varieties (Padulosi and Ng, 1997).

Cowpeas grow under a wide range of conditions but well in areas that have a temperature between 20 – 34 ^{o}C. Cowpea requires precipitation of about 600 mm up to 1500 mm per year. The crop does not like water logged soils but they grow on a wide variety of soils provided they are well drained. Cowpea can grow on poor acidic soil with the pH 5.5 – 6.5. They are sometimes grown as soil improvers (Purseglove, 1968).

It is estimated that 3.7 million tons of cowpea is produced every year from 8.7 million

hectares of land. Africa is the largest producer of cowpea (Tarawali *et al*., 1997) and contributes 87 % of the world's cowpea production area from 6 million hectares, followed by America with 10 % and Europe and Asia share 3 % (Mortimore *et al.*, 1997; Pereira *et al*., 2001). In Africa, the north western region is the largest producer and produces 2.6 milliontons of cowpea. Nigeria is a major cowpea producer and it accounts for 2.2 million tons per year from 5.1 million hectares (Brink and Belay, 2006). The low resource farmers dominated the cowpea production in sub-Saharan Africa and the majority of them grow cowpea in traditional mixed cropping production (Kitch *et al.*, 1997). Cowpea is grown in a pure stand or intercropped with maize, sorghum, rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L.), millet, peanut, cotton (*Gossypium hirsutum* L.), bambara groundnuts (*Voandzeia subterranea* L. Verdc.), cassava (*Manihot esculanta* Crantz), yams (*Dioscorea rotundata* L.) and sesame (*Sesamum indicum* L.) or in a mixture of these crops (Kitch *et al.*, 1997, Mortimore *et al.*, 1997; Tarawali *et al*., 1997; Terao *et al*., 1997). The fodder type is grown in high densities (Mortimore *et al.*, 1997).

2.1.2.2 Morphology of cowpea

Cowpea is described as an annual or perennial crop. They have a well developed tap root with many lateral roots near the surface. The lateral roots have large nodes that are often collected in groups. The growth habit of cowpea varies and may be erect, trailing, climbing or bushy, usually indeterminate under favourable conditions (Purseglove, 1968; Kay, 1979; Fox and Yaung, 1982; Summerfield *et al*., 1974). Cowpeas have smooth, alternate and trifoliate leaves (Figure 2.2) that exhibit considerable variation in size (6-16 x 4-11 cm) and are linear-lanceolate to ovate in shape (Purseglove, 1968; Kay, 1979; Fox and Yaung, 1982). They are usually dark green in colour and the leaf petiole is 5 – 25 cm. The flowers are self-pollinated and the corollas may be white, dirty yellow, pink, pale blue or purple in colour (Cobley and

Steele, 1976; Summerfield *et al*., 1974). The fruit is an erect, linear-cylindrical smooth or slightly warty pod measuring 5-12 mm, depending on the variety (Cobley and Steele, 1976). Pods contain 8-20 seeds, which vary considerably in size, shape and colour. The seed are between 3-6 mm long and the weight is 10 -30g/100 seed (Purseglove, 1968; Kay, 1979; Fox and Yaung, 1982).

Figure 2.2: Cowpea plants (Masangwa, J., 2011).

2.1.2.3 Importance of cowpea

As a food crop, cowpea is cultivated mainly for home consumption where grain and leaves are sources of high quality protein and vitamins (Kitch *et al.*, 1998; Singh *et al.,* 2003). Cowpea tender leaves and green pods are sometimes consumed as vegetables (Brink and Belay, 2006). Cowpea seed contains on average 22-25 % protein; 56 – 66 % carbohydrate; 1.3 – 1.5 % fats; 3.9 % fibre; 3.6% ash; calcium 76 mg/100 g; iron 5.7 mg/100 g; vitamin A 40 iu/100 g; thiamine 0.92 mg/100 g; riboflavin 0.18 mg/100 g; niacin 1.9 mg/100 g; folic acid 0.15-0.16 mg/100 g and ascorbic acid 2 mg/100 g. The cowpea leaves have 8 % carbohydrates, 4.7 % proteins, 2 % crude fibre and 0.3 % fats (Purseglove, 1968; Kay, 1979).

The dry seeds of cowpea are sometimes used as a substitute for coffee (Purseglove, 1968).

Cowpea is a source of income to farmers after selling of grain and processed cowpea foods. Income is also realized by selling haulms as cowpea fodders to livestock farmers (Quin, 1997). As a soil improver, cowpea improves soil fertility by symbiosis with nodule bacteria (rhizobia), which reduce atmospheric nitrogen (N_2) into the compounds assimilated by the host plant. The crop can fix up to 150 kg N/ha into the soil (Summerfield *et al*., 1974; Singinga *et al*., 2000) and the fixed nitrogen can be utilized by the non-leguminous crops in the case of an intercropping systems or after rotation in the subsequent year. Cowpea also improves soil fertility by decomposition of its residues after harvesting (Carsky *et al*., 2001).

Cowpea varieties that have the spreading and indeterminate or semi-determinate bushy growth provide the ground cover that suppresses weeds and also provides some protection against soil erosion. The crop also causes suicidal germination of the parasitic plant, *Striga hermonthica* (Del.) Benth. seeds, which infest cereals (Quin, 1997).

Cowpea is also used as a fodder crop (Purseglove, 1968; Mortimore *et al.*, 1997; Quin, 1997; Brink and Belay, 2006) and research has shown that livestock fed with cowpea haulms as a supplement feed have increased weight gain as compared with those that are not given cowpea as haulms (Singh *et al*., 2003). The green haulms are sometimes cut and mixed with dry cereals for stall feeding (Tarawali *et al.*, 1997).

Kritzinger (2004) reported that cowpea has many medicinal properties as it is used to heal open wounds, abscesses, tumour and sores. Roots of cowpea are used for the relief of menstrual pains, chest pains and also to cure epilepsy (van Wyk and Gericke, 2000).

2.1.2.4 Field disease constraints of cowpea

Fungal diseases are a major problem during the rainy season where insect pests and viral diseases damage the crop during the dry season (Brink and Belay, 2006). The crop succumbs

to diseases caused by common soil-borne fungi such as *Pythium ultimum* Trow, *Rhizoctonia solani* Kühn. and *Fusarium solani* (Mart) Sacc. (Aveling and Powell, 2005). The foliar diseases such as bacterial canker (*Xanthomonas* spp.), rust (*Uromyces phaseolus* var. *vignae*), charcoal rot (*Sclerotium rolfsii* Sacc.), anthracnose (*Colletotrichum*) fungi, Green Mottle Virus and Tobacco Mosaic Virus attack the crop (Kay, 1979).

2.1.3 Anthracnose disease of common bean and cowpea

Than *et al*. (2008) reported that the name anthracnose was derived from a Greek term that means "coal" and they are plant diseases characterized by very dark, sunken lesions that contain spores. Anthracnose is the most serious seed-borne fungal disease of common bean and cowpea. It is common in Africa where farmers like to exchange seed with their neighbours or relatives (Nkongolo, 2003; Kapila, 2008). It is reported that bean anthracnose was first registered in France in 1843 (Martínez-Pacheco *et al*., 2009) and cowpea anthracnose in South Africa in 1997 (Smith and Aveling, 1997). The common bean anthracnose is caused by *Colletotrichum lindemuthianum* (Sacc. and Magn.) Bri. and Cav. (Balardin *et al.*, 1997) and that of the cowpea is caused by both *C. dematium* (Fr.) Grove and *C. lindemuthianum* (Adebitan *et al*., 1992; Allen *et al.*, 1996; Emechebe and Florini, 1997; Vanderborght and Baudoin, 2001). However, the molecular, morphological and antigen differences that exist between the anthracnose pathogens of cowpea and *Phaseolus* beans suggested that the cowpea anthracnose pathogen is probably a form of a *C. gloeosporiodes* (Penz) Penz and Sacc (Emechebe and Florine, 1997). Anthracnose can affect all the aerial parts of bean and cowpea plants at all the growth stages (del Rio and Bradley, 2002). The early symptoms of the disease are lesions easily identified as small, elongated, dark brown areas that occur on petioles and follow the vein patterns of leaves (Tu, 1988). In later stages of disease development lesions can be observed on both sides of the leaf. Anthracnose

disease usually produces small round cankers with a sunken centre on pods (del Rio and Bradley, 2002). The infection can penetrate and infect the newly forming seeds and the infected seed may be shrivelled, discoloured and have dark brown to black cankers (del Rio and Bradley, 2002). The bean anthracnose may cause severe economic losses up to 100 % (Allen, 1983, Pastor-Corrales and Tu, 1989) while 50 % losses have been reported in cowpea (Emechebe and Florini, 1997). The severity of anthracnose disease caused by *C. dematium* is high where the temperatures are as high as 30 ^{o}C (Pakela *et al.*, 2002) whereas that caused by *C. lindemuthianum* is high at temperatures between 15 and 24 ^{o}C (Williams, 1975; O' Connell *et al.*, 1985). High humidity of over 95 % or free water on leaves and early infection of seedlings of less than three week old of both crops by the anthracnose disease leads to severe damage (Pastor-Corrales and Tu, 1989; Pakela *et al.*, 2002). Anthracnose was and still is considered the most important disease in bean producing areas and is regarded as a major hinderance to the improved health, prosperity and stability of people in the rural areas of Africa and Latin America (Dron and Bailey, 1999). Several races of the *Colletotrichum* species have been reported from many countries (Batista and Chaves, 1982; Emechebe and Florini, 1997). The races differ in form, growth rate and colony appearance, but also in conidial production and pathogenicity (Batista and Chaves, 1982). Some races of the *Colletotrichum* pathogen are resistant to the effective fungicide asreported by Emechebe and Florini (1997) suggesting the use of combined different management measures to control the anthracnose disease.

Figure 2.3: Anthracnose diseased cowpea (left) and bean (right) pods

2.2 LITERATURE REVIEW OF *COLLETOTRICHUM* SPECIES

Colletotrichum Corda is one of the most important genera of pathogenic fungi worldwide causing economically important diseases of cereals, grasses, legumes, vegetables and perennial crops including trees (Dron and Bailey, 1999; Horvath and Vargas, 2004.; Wharton and Dieguez-Uribeondo, 2004; Turechek *et al*., 2006). *Colletotrichum* was originally spelt as *Colletothricium* in 1831 but was later changed to its present spelling in 1837 (Sutton, 1966). *Glomerella* is the anamorph of *Colletotrichum* (Than *et al*., 2008). The fungi attack all parts of the plant, at all stages of development, from seedlings to mature plant and seed, causing disease symptoms commonly known as anthracnose (Bailey and Jeger, 1992).

Although *Colletotrichum* spp. are known as plant pathogens recent reports have indicated that there are some species of *Colletotrichum* that are associated with human and animal diseases. These infections have mostly been found in immunosuppressed people (Sutton, 1999; Cano *et al.,* 2004). Mendiratta *et al*. (2005) reported that *C. dematium* is responsible for corneal

ulcers called keratitis in humans. Other *Colletotrichum* species that are also reported to cause infection in humans are *C. gloeosporioides*, *C. coccoides* (Wallr.) S. Hughes and *C. graminicola* (Cesati) Wilson. (De Hoog *et al.*, 1995; Cano *et al.*, 2004). *Colletotrichum* spp. are also reported to be associated with animals and fish (Manire *et al.*, 2002; Mendiratta *et al*., 2005).

2.2.1 *Colletotrichum lindemuthianum*

Martínez-Pacheco *et al*. (2009) reported that *C. lindemuthianum* was designated with several names such as *Gleosporium lindemuthianum* (1878), *Septoria legunimun* (1882), *Septoria leguminum* var. *phaseolorum*, *C. lindemuthianum* (1889), *C. lagenarium* (1893), *Gleosporium lindemuthianum* (1894) and *Glomerella lindemuthiana* (1993). The pathogen has been identified as a fungus that presents imperfect and perfect forms, which have been denominated by *C. lindemuthianum* and *Glomerella cingulata* (Stonem.) Spauld. et Schrenk f.sp. *phaseoli*, respectively (Martínez-Pacheco *et al*., 2009).

Colletotrichum lindemuthianum is reported to cause anthracnose of common bean and cowpea (Balardin *et al.,* 1997; Smith, 1997). Brown lesions along the leaf veins of the lower and upper leaf surface, eye shaped lesions on stems and sunken lesions on the pods are symptoms associated with this pathogen (Tu, 1988; Baudoin *et al.*, 2001). Colonies are initially light grey, but darken with age and the production of compact aerial mycelium. Conidia of *C. lindemuthianum* are straight, short, and cylindrical with regular edges, 5-7 x 5-6 µm, and pale to cinnamon or dark brown and produce pale salmon to honey-coloured masses of conidia (Sutton, 1992). The conidia are borne in acervuli, which are roundish or elongated. Acervuli are 300 µm in diameter and have setae that are formed from occasional cells (Pastor-Corrales and Tu, 1989). Setae are numerous, long, slender, dark brown to black (Sutton, 1992).

2.2.1.1 Interaction with plant hosts

Colletotrichum lindemuthianum usually overwinters either in seed or infected crop residues. The pathogen can be viable for almost two years in the residues or infected seed but can be viable for five years in the infected pods (Tu, 1988; Pastor-Corrales and Tu, 1989). The survival longevity of *C. lindemuthianum* is contingent upon environmental factors of which moisture plays a critical role (Pastor-Corrales and Tu, 1989). The conidia are dispersed by wind, splashing rain, insects, animals and humans (Tu, 1988; Pastor-Corrales and Tu, 1989). Conidia attach themselves to the plant surface before germination and produce germ tubes (Perfect *et al*., 1999). The germination of conidia occurs six to nine hours under favourable conditions (Pastor-Corrales and Tu, 1989). Other workers however reported that conidia of *C. lindemuthianum* germinate within 12 h post inoculation (hpi) on cowpea stems (Bailey *et al*., 1990) and 18 hpi on French bean (O' Connell *et al*., 1985). The appressoria are formed and reach maximum values in 24 to 48 hpi (Skipp and Deverall, 1972). The appressoria wall is impregnated with melanin which enables it to have a penetration force of about 16.8 ± 3.2 µm at the penetration peg to penetrate through the tissue cuticle (Skipp and Deverall, 1972). *C. lindemuthianum* is a hemi-biotrophic fungus (Perfect *et al*., 1999; Mendgen and Hahn, 2002) and it grows as a biotroph with primary intracellular hyphae for one or a few days (50 - 74 hpi) after penetrating the cuticle and cell wall without killing the host cells (Skipp and Deverall, 1972). The primary intracellular hyphae are enclosed in the extra-cytoplasmic matrix and are connected to the plant apoplast (Mendgen and Hahn, 2002). The killing of host cells and proliferation of necrotrophic growth starts soon after the formation of secondary narrow hyphae (Herbert *et al*., 2002; Mendgen and Hahn, 2002). The narrow necrotrophic hyphae are formed at the end of the biotrophic phase (Perfect *et al*., 1999; 2001) and these hyphae produce a large amount of cell-wall-macerating enzymes, which degrade cell walls and also reduce the host defense reaction (Perfect *et al*., 2001; Yokoby *et al*.,

2001). Mims and Vaillancourt (2002) reported that the anthracnose lesions appear during the necrotrophic phase of the *Colletotrichum* disease cycle. According to Skipp and Deverall (1972), the disease symptoms (lesions) appear 5 - 6 days post inoculation. The disease caused by these fungi tends to be more severe in crops grown in monoculture where it spreads rapidly under wet conditions at temperatures between 15 – 24 ^{o}C (Williams, 1975; O' Connell *et al.*, 1985). High humidity of over 95 % or free water is required during all the stages of conidium germination (Pastor-Corrales and Tu, 1989). Different temperatures and cultivars significantly affect the disease severity (Emechebe, 1981). There are seven known races of *C. lindemuthianum*. Some races of *C. lindemuthianum* are not compatible with some cultivars of *P. vulgaris* and the incompatibility of *C. lindemuthianum* with a particular resistant cultivar of *P. vulgaris* is characterized by early death at the point of penetration (Skipp and Deverall, 1972; O'Connell *et al*., 1985). The inhibition of further growth of the pathogen in the penetrated tissue and limiting lesion growth were reported by Pakela (2003). The high correlation between the hypersensitive response and race-cultivar specificity as indicated by early hypersensitive response in the incompatible cultivars does not occur in compatible interactions as shown in the Figure 2.1 (Pakela, 2003).

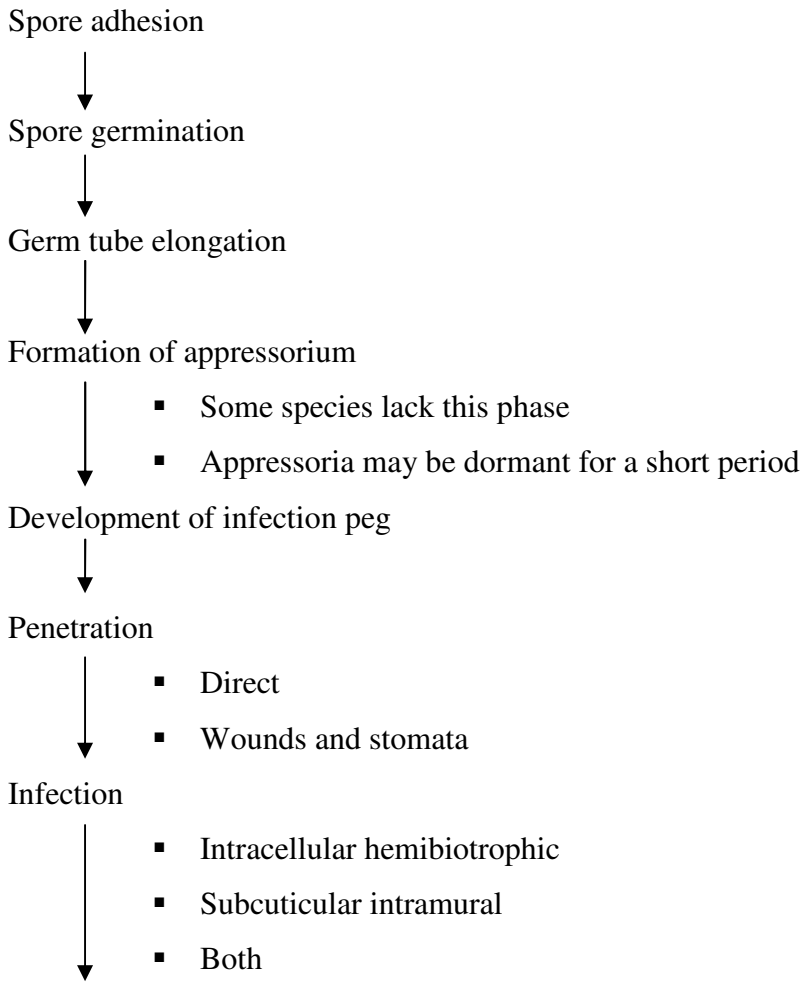

Figure 2.1: Morphological events in the infection process of *Colletotrichum* species (adapted from O'Connell *et al*., 1985; Smith and Aveling 1999 and Pakela, 2003).

2.2.2 *Colletotrichum dematium*

Colletotrichum dematium var. *truncata* was first known as *Sphaeria trichella* F. and later as *Sphaeria dematium* in 1823. It was then suggested that papillate *Sphaeria* species including *S. dematium*, should be placed in *Vermicularia* Tode. *Sphaeria dematium* was placed in *Colletotrichum* in 1918. A number of species in the genus *Colletotrichum* have been placed under *C. dematium* either as synonyms, variations, form or *formae speciales* based on

morphological features such as conidial and host specificity (Koch *et al.*, 1989). The colonies of *C. dematium* on potato dextrose agar (PDA) are cottony or felty towards the center, pale to deep mouse-grey (Gourley, 1966), alternating with course zones of deep mouse grey to black acervuli. Acervuli develop on PDA, cushion-like to conical, stalked with age, and darkly pigmented with setae, which project above whitish to pale salmon conidial masses. Setae are wide at the base, abundant, black, erect, smooth-walled and 60-200 x 4-7.5 µm. Conidia are hyaline, smooth-walled, aseptate, curved fusiform, gradually tapered to each end and 18-32 x 2-4 µm (Baxter *et al.*,1983; Sutton, 1992; Mathur and Kongsdal, 2003).

2.2.2.1 Interaction with plant hosts

The host range of *C. dematium* varies widely but it infects leguminous crops although it occurs on some non-leguminous hosts. *Colletotrichum dematium* was once described as a pathogen of mature tissue (Sutton, 1992). Conidial germination begins six hours after inoculation. The germ tubes develop from random points on the conidia but the majority form one germ tube (Smith, 1997). The appressorium is bulbous and sessile, developing from any point on the conidium. Germ tubes and appressoria are formed directly above epidermal cells or stomata, with no specific orientation to stomata, whether open or closed (Smith, 1997). Penetration by infection tubes occurs directly through the plant cuticle and cell wall. The infection vesicles are formed within the epidermal cells 20 hpi. The rapid growth of hyphae commences 20 – 30 hpi and this proceeds until the initial infected cells are packed with convoluted mycelium (Smith, 1997). The hyphae grow extensively inter-and intracellularly passing through cell walls and within 48 hpi light brown lesions appear on the stem (Smith, 1997). The tan brown discolouration stem symptoms later spread, and then become dark purplish-brown and sunken lesions (Emechebe and Florini, 1997; Smith and Aveling, 1997). *Colletotrichum dematium* also produces phytotoxins in the border of anthracnose lesions that

induces necrosis (Yoshida *et al*., 2000). Sunken, necrotic lesions and small, black acervuli are visible on diseased stems. The severity of anthracnose disease caused by *C. dematium* is high where the temperatures are as high as 30 ^{o}C (Pakela *et al*., 2002). Roy (1982) reported that *C. dematium* causes seed rot, pre-emergence and post-emergence killing of seedlings.

2.3 LITERATURE REVIEW OF BOTANICALS

2.3.1 Sweet potato (*Ipomoea batatas* (L) Lam.)

2.3.1.1 Origin and distribution

Ipomoea batatas (L) Lam. (Figure 2.4) is placed in the family Convolvulaceae (Cobley and Steele, 1976). Sweet potato is an important food crop, which is widely grown in tropical, subtropical, and warm temperate regions. Tropical America is the centre of origin of sweet potato (Cobley and Steele, 1976). Based on the analysis of morphological characters of sweet potato and the wild *Ipomoea* species, the centre of origin of *I. batatas* was thought to be between the Yucatan Peninsula of Mexico and the mouth of the Orinoco River in Venezuela (Austin, 1987). The highest diversity revealed by the use of molecular markers provides evidence that Central America is the primary centre of origin, considering the richness of the wild relatives of sweet potato (Srisuwan *et al*., 2006). The voyage of Christopher Columbus resulted in the introduction of sweet potato to Western Europe from the West Indies and around the 16th Century, this crop was then introduced to Africa, India, South East Asia and East Indies by the Portuguese explorers (Cobley and Steele, 1976; Srisuwan *et al*., 2006).

2.3.1.2 Uses of sweet potatoes

Sweet potato tubers are eaten as a source of carbohydrates, fourth from maize, rice and cassava. The crop is the seventh important crop in the world (Carvalho *et al.*, 2010). Sweet potatoes have many medicinal values. The leaves are used as a remedy for diabetes and the

white skin of the tubers were also reported to have anti-diabetic activities (Watt and Breyer-Brandwijk, 1962). Mazza *et al*.(2007) reported that the sweet potato leaves have medicinal value such as anti-diabetic and anti-bacterial activities due to the presence of a high content of flavonoids such as anthocyanins and phenolic acids (Kaur and Kapoor, 2001). The boiled roots of sweet potatoes are used in the Phillipines to treat diarrhoea (Anonymous, 2011a). Sweet potatoes are also reported to possess some chemicals responsible for a vasorelaxation mechanism of action, as well as the antioxidant and anticancer activities (Anonymous, 2010a and 2011a). According to Elwell and Maas (1995), a solution of sweet potato leaves have fungicidal properties and they speculated that the leaves can be used to control rice brown leaf spot, rice blast fungus and other fungi.

Figure 2.4: *Ipomoea batatas* (L) Lam. (Masangwa, J. 2010)

2.3.2 Garlic (*Allium sativum* L.)

2.3.2.1 Origin and distribution

Garlic (*Allium sativum* L.) is a spice that is cultivated and sold in different parts of the world (Figure 2.5). It is a biennial or annual bulbous herb. Van Wyk (2005) reported that garlic is locally called suan (Chinese), ail blanc (French), knoblauch (German), aglio (Italian),

gaarikku (Japanese) and ajo (Spanish). Garlic is among the oldest known horticultural crops and it is suspected to have originated in the Middle East or central Asia (van Wyk and Wink, 2004). In the Old World, Egyptian and Indian cultures referred to garlic 5000 years ago and there is clear historical evidence for its use by the Babylonians 4500 years ago and by the Chinese 2000 years ago (Simon, 2004). Some writings suggest that garlic was grown in China as far back as 4000 years ago. Earlier in history garlic grew wild over a much larger region and wild garlic may have occurred in an area from China to India to Egypt to the Ukraine (Simon, 2004). This region where garlic has grown in the wild is referred to as its "center of origin" since this is the geographic region where the crop originated and the only place where it flourished in the wild (Simon, 2004).

2.3.2.2 Uses of garlic

Garlic is grown as food condiments. It is also cultivated as a source of income to farmers and the middlemen. According to Brown (2002) and van Wyk and Wink (2004), garlic is used as remedy against the common cold, influenza, whooping cough, gastroenteritis and dysentery. It is also used to treat high blood lipids and in the prevention of age-related vascular changes. Van Wyk and Wink (2004) reported that garlic has diaphoretic, expectorant, antiviral, antispasmodic and antiseptic properties. The *in vitro* studies by Iwalokun *et al.* (2004) showed that aqueous garlic extracts inhibited the growth of multidrug-resistant gram-positive and gram-negative human bacterial pathogens and *Candida* species.

Garlic is also reported to have bactericidal, fungicidal, insecticidal, anti-feedant, nematicidal and repellant action when sprayed on crops (Elwell and Maas, 1995). Volatile compounds of crude aqueous extracts of garlic bulbs inhibit germination of microconidia and hyphal extension in *Fusarium oxysporum* f.sp. *lycopersici* (Sacc) Snyder and Hans., *Magnaporthe grisea* (Hebert) Barr., *Botrytis cinerea*, *Alternaria brassisicola* (Schwein.)

Wiltshire, *Plectosporium cucumerina (*Lindf.) W. Gams and *Phytophthora infestans* (Mont.) De Bary (Taraq and Magee, 1990; Curtis *et al*., 2004). According to Saniewska (1992), garlic clove homogenate and ajoene inhibit mycelium growth of *Stagonospora curtisii* (Berk) Sacc. According to Abdulrahman and Aba Alkhali (2005), Wilson *et al*. (2007) and Shovan *et al.* (2008), garlic extracts inhibit the growth of *B. cinerea*, *C. dematium*, *F. oxysporum* f. sp. *lycopersici* and *R. solani*.

Figure 2.5: *Allium sativum* bulbs (http://www.motherearthnews.com/uploadedImages /Blogs/Grow_It!/garlic-bulbs-325.jpg). Accessed on 30 December 2011.

2.3.3 Pawpaw (*Carica papaya* L.)

2.3.3.1 Origin and distribution

Carica papaya L. (Figure 2.6) belongs to the family Caricaceae (Cobley and Steele, 1976). The wild form of pawpaw seems to have an entirely Central American distribution, and a Central American centre of domestication for pawpaw was reported by Purseglove (1974). Cobley and Steele (1976) reported that Central America is the centre of origin of pawpaw, however, Aradhya *et al*. (1999) reported that others suggested a South American origin, but all pawpaws in South America appear to be introduced domesticates or feral plants escaped from cultivation (Morshidi, 1996). Pawpaw was introduced to the Philippines by Spaniards in

the mid-16th century and from there it was introduced to India, Africa and the rest of the tropical and subtropical world (Purseglove, 1974).

2.3.3.2 Uses of pawpaw

Pawpaw fruit is a good source of dietary fiber, folate, vitamin A, C and E. The fruit also has calcium, iron, riboflavin, thiamine and niacin (Anonymous, 2011b). Pawpaw leaves are used as a meat tenderizer and are also eaten as vegetables (Watt and Breyer-Brandwijk, 1962). Smoking of pawpaw leaves is reported to relieve asthma (Watt and Breyer-Brandwijk, 1962). Pawpaw leaf and bark extracts are reported to be employed as a remedy to mouth sores and toothaches by some cultures around the world (Anonymous, 2011b). According to Oguntola (2010), a pawpaw and mango (*Mangifera indica* L.) leaf mixture is used as a cure to treat malaria in Nigeria. Watt and Breyer-Brandwijk (1962) reported that pawpaw leaves are also a remedy for beriberi. Van Wyk and Gericke (2000) reported that a root infusion of pawpaw is used as a remedy for gonorrhoea in Mozambique and syphilis in east Africa (Watt and Breyer-Brandwijk, 1962). Roots are also used as a remedy for kidney and bladder problems (Watt and Breyer-Brandwijk, 1962). The fruit-pulp is used as a remedy for warts. The fresh seeds cause an abortion if eaten by a pregnant woman (Watt and Breyer-Brandwijk, 1962). Pawpaw crude latex is applied as in injection therapy in damaged intervertebral cartilage and is also applied to the skin to clean wounds (van Wyk and Wink, 2004). The bark of the pawpaw tree is used as remedy for venereal diseases (Watt and Breyer-Brandwijk, 1962). Unripened pawpaw fruit have properties of reducing blood sugar (Egwins, 2005) indicating that they can be used to control diabetes.The latex is reported to have antifungal properties and is used by the Indians of New Guinea to treat fungal infections of the skin (Draelos, 2001). Giordani *et al.* (1996) also reported that *C. papaya* latex sap inhibits the growth of *Candida albicans* (Robin) Berkhout under *in vitro* conditions

Apart from controlling human diseases studies have also showed that extracts of pawpaw leaves can be used to control plant diseases. Taiga *et al*. (2008) reported that pawpaw extract inhibited the growth of *F. oxysporum* mycelium. Studies by Amadioha (1999) showed that cold and hot water leaf extracts of papaya are effective in reducing the growth of powdery mildew fungi *in vitro* and in reducing the spread of powdery mildew disease on pepper (*Capsicum annuum*) plants. Nwachukwu and Umechuruba (2001) reported that the extract of pawpaw leaves also reduces the incidence of seed-borne fungi of African yam bean seed.

Figure 2.6: *Carica papaya* L. *http://www.tradewindsfruit.com/papaya2.jpg*. Accessed on 30 January 2012.

2.3.4 *Agapanthus caulescens* Spreng.

2.3.4.1 Origin and distribution

Agapanthus (Figure 2.7) belongs to the Agapanthaceae family (Pienaar, 2001; Brickell, 2003) and is believed to have originated from South Africa (van der Una, 1971). The botanical name, *Agapanthus*, is derived from the Greek "*agape*" (love) and "*anthos*" (flower) (Pienaar, 2001). *Agapanthus* is commonly called blue lily or lily of the Nile or African tulip or African

lily (English), bloulelie (Afrikaans), isicakathi (Xhosa), leta-la-phofu (Sotho) and ubani (Zulu) (van Wyk *et al.*, 1997). *Agapanthus caulescens* is a perennial rhizomatous plant (Pienaar, 2001) that is grown in gardens around the homestead and in parks. *Agapanthus* grows naturally in South Africa, Swaziland, Lesotho and Mozambique (Anonymous, 2011c). The plant is also grown in Europe, North and South America, Australia and New Zealand (WIPO, 2007).

2.3.4.2 Uses of *Agapanthus*

Agapanthus is grown as an ornamental in the garden for flowers. *Agapanthus* is used by some South African traditional healers as a remedy for heart disease, paralysis, cough, colds and chest pains (Anonymous, 2010b and 2011c). The plant is reported to be used during pregnancy to induce delivery (Watt and Breyer-Brandwijk, 1962; van Wyk *et al*., 1997). The decoction of the roots of *Agapanthus* is given to a newly born baby before being put to the breast for the first time (Watt and Breyer-Brandwijk, 1962). A lotion made from crushed roots is applied to a Sotho new-born to ensure strength (Watt and Breyer-Brandwijk, 1962). *Agapanthus* also has anti-inflammatory, antioedema, antitussive and immunoregulatory properties (van Wyk *et al*., 1997).

Studies by Pretorius *et al*. (2002) and Tegegnea *et al*. (2008) revealed that *Agapanthus* has antifungal activities against phytopathogens such as *B. cinerea*, *R. solani*, *Verticillium dahlia* Kleb., *Botryosphaeria dothidea* (Moug.:Fr.) Ces & De Not., *F. oxysporum*, *Sclerotium rolfsii* Sacc., *Pythium ultimum*, *Sporisorium cruenta* Kuhn; Potter. and *Sporisorium sorghi* Link; Clinton. Other studies showed that the leaf extracts of *Agapanthus* possess antifungal and antibacterial activities against some human pathogens (Fawole *et al.*, 2009).

Figure 2.7: *Agapanthus caulescens* Spreng.

(www.plantzafrica.com/plantab/agapancaul.htm).Accessed on 16 August 2011.

2.3.5 *Chlorophytum comosum* (Thunb.) Jacq.

2.3.5.1 Origin and distribution

Chlorophytum comosum (Thunb.) Jacq. cv. variegatum. (Figure 2.8) is in the Anthericaceae family (Brickell, 2003). Hen and chicken and spider plant (English), hen en kuiken (Afrikaans) and Phamba (Zulu) (Reymond, 2002) are common names of *C. comosum*. Matsushita *et al.,* (2005) and Kaushik (2005) reported that *C. comosum* originated from South Africa. *Chlorophytum comosum* is an evergreen perennial herb, which has a base concentration of leaves that grow up to 300 mm long (Gathe and Watson, 2008). The leaves are alternately arranged and are variegated with a white margin. The leaves are flat (grass-like) and have no hairs. *Chlorophytum* produces hermaphrodite fertile flowers, which result into non-fleshy fruits (Gathe and Watson, 2008).

2.3.5.2 Uses of *Chlorophytum comosum*

Chlorophytum is grown for ornamental use around the homestead and in parks in South Africa. According to Aberoumand and Deokule (2008; 2009; 2010), *C. comosum* is consumed as a vegetable in Iran.

All species of *Chlorophytum* have medicinal values (Raghavendra *et al.*, 2006; Dabur *et al*., 2007). *Chlorophytum borivilianum* Santapau and Fernandes is reported to possess vast medicinal values such as anti-diabetic, anti-cancer, hepato-protective, anti-malarial and sedative properties. The roots of *C. borivilianum*, *C. laxum* R. Br. and *C. tuberosum* (Roxb.) Bak. are also used as a drug for diarrhoea and dysentery (Dabur *et al.,* 2007). *Chlorophytum borivillianum* is used as a general tonic for strength and vigour effects (The Wealth of India, 1992) and its combination with other medicinal plants has anti-epileptic activity (Balamurugan *et al.*, 2009). Kaushik (2005) and Matsushita *et al.* (2005) reported that *C. comosum* is used as a traditional medicine to treat bronchitis, fractures and burns. The infusion of the tubers of *C. comosum* is administered to new-born infants as a purgative by the Xhosa and is also taken by women immediately after giving birth (Watt and Breyer-Brandwijk, 1962). The medical research has shown that *C. comosum* has anti-tumor properties and can induce apoptosis in human cell lines (Matsushita *et al.*, 2005). Pretorius *et al*. (2002) found that the plant has antifungicidal activities against *P. ultimum*, a phytopathogen. Raghavendra *et al.* (2006) reported that *Chlorophytum* is used as agricultural fungicide as a seed treatment.

Figure2.8: *Chlorophytum comosum* (Masangwa, J. 2009).

2.3.6. *Syzygium cordatum* Hochst ex. C. Krause

2.3.6.1 Origin and distribution

Syzygium cordatum Hochst.ex C. Krause is from the family Myrtaceae (Figure 2.9). The generic name *Syzygium* is derived from the Greek word meaning "coupled", an illusion to the paired branches and leaves. *Cordatum* comes from a Latin word "cordatus", which means heart-shaped in reference to the heart-shaped base of the leaves. Its common names are: waterbessie (Afrikaans), umdoni (Zulu), montlho (Sotho), myamayu, mlati (Swazi), water berry, water wood and water tree (English) (van Wyk *et al*., 1997; Brink, 2008), nyowe (Chichewa), katope, mchisu and nsinika (Tonga) (Burrows and Willis, 2005). *Syzygium cordatum* is an evergreen tree, which grows to a height of 8 -15 m. The leaves are elliptic to circular, bluish green on top and a paler green below but young leaves are reddish in colour (Palgrave, 2002). The creamy white to pinkish 2 – 2.5 cm diameters fragrant flowers are borne in branched terminals and have numerous fluffy stamens and produce abundant nectar (Pooley, 1997; Palgrave, 2002). The fruits are oval berries, red to dark-purple when ripe (Pooley, 1997; Palgrave, 2002; Brink, 2008). According to Burrrows and Willis (2005) and

Brink (2008), *S. cordatum* is distributed from the Democratic Republic of Congo eastwards to Kenya and southwards to South Africa.

2.3.6.2 Uses of *Syzygium cordatum*

The *S. cordatum* fruits (Figure 2.9) are food for the wild birds, monkeys and are also eaten by humans. The fruits are also used in alcohol making (Palgrave, 2002). The wood is used for timber and fire wood (Palgrave, 2002). The flowers of *Syzygium* trees attract bees hence they are used in apiculture. The extracts from the bark of the tree are also used in dye making (van Wyk and Gericke, 2000; Jansen and Cardon, 2005). The tree bark is dried and made into powder, which is used as fish poison for catching fish (Brink, 2008).

All of the *Syzygium* spp. have been reported to have medicinal values and have potential to control many human diseases (Noomrio and Dahot, 1996; Geyid *et al*., 2004; Musabayane *et al.*, 2005; Krishnaraju *et al*., 2006; Kumar *et al*., 2009). In Africa, *S. cordatum* is used as a remedy for respiratory ailments, tuberculosis, diarrhoea and stomach ache (van Wyk *et al*., 1997; Sibandze, 2010). Leaf extracts and the bark are used as a cure for diarrhoea (van Wyk and Gericke, 2000; Sibandze, 2010). A decoction of roots and bark are used as a remedy for indigestion and malaria whereas the infusion of roots and stems are taken to treat coughs (Brink, 2008). Pretorius *et al.* (2002) found that *S. cordatum* extracts have antifungal activities against *P. ultimum.*

Figure 2.9: *Syzygium cordatum* tree and its fruits (Masangwa J., 2009).

2.4 SUMMARY OF LITERATURE REVIEW

This literature review covered aspects such as the origin, world production status and systems of common bean and cowpea crops. The botany, importance and the field disease constraints on production of common bean and cowpea were also reviewed. The field disease constraints highlighted the major diseases that greatly reduce the yields of the two crops.

Anthracnose disease and the *C. lindemunthianum* and *C. dematium* pathogens that cause anthracnose of common bean and cowpea, respectively, were reviewed. The history of *C. lindemuthianum* and *C. dematium* was covered. Furthermore, the interaction of the two *Colletotrichum* spp. mentioned above with common bean and cowpea was discussed. This included the dissemination of the pathogen from diseased to clean bean and cowpea plants, attachment, germination and all other processes that happen to initiate the anthracnose disease.

The literature review encompassed the origin, distribution and uses of *A. caulescens*, *A. sativum*, *C. papaya*, *C. comosum*, *I. batatas* and *S. cordatum*. Emphasis was placed on the

medicinal uses, antifungal and bactericidal activities of the above mentioned botanical plants on human and plant pathogens. The botanicals literature review has revealed that the above listed plants have fungicidal and bactericidal activities against human and phytopathogenic pathogens; however, it is evident that little work has been done on fungicidal properties of the plants described against *Colletotrichum* spp. and anthracnose of common bean and cowpea.

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CHAPTER THREE

IN VITRO SCREENING OF PLANT EXTRACTS FOR ANTIFUNGAL ACTIVITIES AGAINST *COLLETOTRICHUM* SPECIES OF COMMON BEAN (*PHASEOLUS VULGARIS* L.)AND COWPEA (*VIGNA UNGUICULATA* L. WALP)

3.1 ABSTRACT

The investigation was initiated with the aim of evaluating the antifungal activities of plant extracts that can be used to control bean and cowpea anthracnose. Acetone, ethyl acetate and water extracts of *Syzygium cordatum* Hochst.ex Krauss, *Chlorophytum comosum* cv. Variegatum, *Agapanthus caulescens* Spreng., *Ipomoea batatas* (L.) Lam, *Allium sativum* L. and *Carica papaya* L. were screened for their antifungal activities against *Colletotrichum lindemuthianum* (Sacc. and Magn.) Bri. and Cav. and *Colletotrichum dematium* (Fr.) Grove var. *truncata* of cowpea and common bean using the agar infusion and microtitre dilution techniques. The water extracts of *C. papaya* and *Syzygium* were active against *C. lindemuthianum* and gave 1.56 mg.ml^{-1} as their minimum inhibitory concentrations (MIC). *Syzygium*, *Allium* and *Chlorophytum* water extracts were active against *C. dematium* and had 3.13, 6.25 and 12.5 mg.ml^{-1} as their MIC. *Allium, Syzygium* and *Agapanthus* acetone extracts gave 0.78, 3.13 and 6.25 mg.ml^{-1} as their MIC against *C. lindemuthianum* and 0.78, 6.25 and 3.13 mg.ml^{-1} against *C. dematium.* The inhibition increased with an increase in concentration except in all *Ipomoea* and *Chlorophytum* (acetone and ethyl acetate) extracts where it promoted the growth of *Colletotrichum*. *Allium*, *Agapanthus*, *Carica* and *Syzygium* extracts showed good antifungal activities against both *C. lindemuthianum* and *C. dematium*. *Ipomoea* extracts exhibited no antifungal activity against *C. dematium* and *C. lindemuthianum*.

Key words: antifungal extracts, *Colletotrichum*, *Phaseolus vulgaris, Vigna unguiculata*

3.2 INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) and cowpea (*Vigna unguiculata* L. Walp) are two important legume crops that are commonly grown in the sub-Saharan region of Africa for food, cash, animal feed and as soil improvers. The seed of the two crops are a cheap source of proteins to the people of this region and their easy storability makes them to be preferred over meat products by smallholder farmers (Msuku *et al*., 2000) who mostly lack cooling facilities like fridges. It has been reported that the two crops also have medicinal properties (Ye *et al*., 2000; Wong and Ng, 2005; Wong *et al*., 2006; Jeng *et al.,* 2009; Lam and Ng, 2010). The cowpea leaf extracts (Kritzinger *et al.*, 2005) and seed extracts of both crops have antifungal activities to some phytopathogens (Ye *et al*., 2000; Wong *et al*., 2006) making them the potential source of agricultural fungicides.

Despite the importance of the two crops, farmers fail to realize maximum potential yields because of several limiting factors; among them the incidence and severity of diseases (Coyne *et al*., 2003; Adebanjo and Bankole, 2004). Anthracnose is one of the common diseases of legumes and is caused by the fungus genus *Colletotrichum*. Common bean anthracnose is caused by *Colletotrichum lindemuthianum* (Sacc. and Magn.) Bri. and Cav. whilst cowpea anthracnose is initiated by both *Colletotrichum dematium* (Fr.) Grove var. *truncata* and *C. lindemuthianum* (Allen, 1983). Anthracnose is a seed-borne disease and under heavy infection of the disease no yields can be realized (Bokosi, 1986). It is also imperative to control anthracnose because the pathogen causing this disease has been reported to be associated with humans and animals (Cano *et al.,* 2004; Mendiratta *et al.,* 2005). For example, *C. dematium* was implicated in corneal ulcer in humans (Mendiratta *et al.,* 2005).

The current major method of controlling anthracnose is the use of synthetic fungicides (Tian *et al.,* 2007) through seed treatment and foliar application. However, many demerits pertaining to their use have been highlighted by several scientists (Falandysz, 2000; Shovan *et al.,* 2008) and there is a need to search for safer fungicides that can be used alternatively.

There are many plants that are toxic to *Colletotrichum*, however, research in this field has focused upon the anthracnose diseases of vegetables and fruits (Raghavendra *et al.,* 2006; Chowdhury *et al.*, 2007; Choi *et al.*, 2008; Nduagu *et al.,* 2008; Mdee *et al.,* 2009) and little has been done on the anthracnose of bean and cowpea (Amadioha, 1999; Amadioha, 2003; Shoven *et al.*, 2008; Akinbode and Ikotum, 2008, Pinto*et al.*, 2010). Hence this work was initiated.

The aim of the investigation was to evaluate plant extracts for antifungal activities against *C. dematium* and *C. lindemuthianum* that could possibly lead to their use for the control of bean and cowpea anthracnose. The minimum inhibitory concentrations (MIC) of different plant extracts extracted by different solvents were also determined.

3.3 MATERIALS AND METHODS

3.3.1 Plant materials

Syzygium cordatum Hochst.ex Krauss (fruits), *Chlorophytum comosum* (Thunb.) Jacq. cv. Variegatum (whole plant), *Agapanthus caulescens* Spreng. (whole plant), sweet potato (*Ipomoea batatas* (L.) Lam) (leaves), garlic (*Allium sativum* L.) (bulbs) and pawpaw (*Carica papaya* L.) (leaves) were used in this study. *Syzygium cordatum*, *C. comosum*, and *A. caulescens* were collected from the Manie van der Schijff Botanical Garden at the University of Pretoria, Pretoria. The sweet potato leaves were collected from the University of Pretoria's Experimental Farm. The garlic bulbs were bought at the Pretoria fresh market and the pawpaw leaves were collected from Durban in the Kwazulu-Natal Province. Samples of the

collected plants were deposited in the University of Pretoria H.G.W.J. Schweickerdt Herbarium and voucher numbers were assigned (Table 3.1).

3.3.2 Preparation of the crude extracts

All the plant materials were air dried in the shade and ground to powder by using a pestle and mortar. Sequential extraction was performed on 1 kg of each plant powder by soaking them in acetone, ethyl acetate and sterile distilled water. The plants were soaked in that order contingent to the polarity of the solvents. The organic solvents were removed using a Büchi Rotavapor (Model R-200, Switzerland) and water plant filtrates were concentrated to powder on a freeze drier (Edwards High Vacuum International, Sussex, England). Different quantities of crude extracts were harvested from different solvents as shown in Table 3.1. All the extracts were then stored in the fridge at ±4^{o}C.

3.3.3 Collection of anthracnose infected plants and isolation of *Colletotrichum* spp.

Anthracnose infected common bean and cowpea plants were collected from Middelburg, Mpumalanga and the Department of Agriculture experimental farm, Nelspruit, Mpumalanga, respectively. Stems and leaves disinfested in 1% sodium hypochloride for three minutes and rinsed three times in sterile distilled water were placed on moist blotters in Petri dishes and on potato dextrose agar (PDA) under continuous UV light for 7 d. *Colletotrichum* spp. were identified using the Common Laboratory Seed Testing Methods for Detecting Fungi (Mathur and Kongsdal, 2003), sub-cultured and stored in a fridge (±4^{o}C) until needed.

3.3.4 Antifungal bioassays

3.3.4.1 Agar infusion technique

The antifungal activity of the extracts was determined by adopting the technique described by

Kritzinger *et al*. (2005) where appropriate amounts of each stock of crude extract was added to 100 ml of PDA before pouring in Petri dishes to yield final concentrations of 0.5, 1.0, 2.5 and 5.0 mg.ml^{-1}. The amended PDA was poured into 65 mm Petri dishes with preset diametrical lines drawn on the bottom plate to identify the centre of the plate. Five millimetre diameter plugs of *C. lindemuthianum* and *C. dematium* from 7-day-old fungal cultures were placed in the centre of the Petri dishes containing PDA amended with extracts, acetone 0.05 ml/ml (v/v), ethyl-0.05 ml/ml (v/v) acetate and Celest® XL (25 gai/L) (fludioxonil and mefenoxam, a synthetic fungicide from Syngenta SA, Midrand). The Petri dishes were then sealed with Parafilm® and incubated at 25°C. Acetone, ethyl acetate and unamended PDA represented the negative control while Celest® XL represented the positive control. The treatments were arranged in a completely randomized design and each treatment was replicated four times. The experiment was repeated twice. Evaluation of Petri dishes was executed by measuring the diameter of fungal growth after 3, 6 and 9 days of incubation (DAI). The formula of Tegegne *et al*. (2008) was used to calculate the percentage inhibition of mycelial growth:

% Inhibition = (dc – dt)/dc x 100

Where dc = average diameter of fungal colony of the negative control (PDA only) and dt = average diameter of fungal colonies grown in the presence of plant extracts or standard fungicides.

The percentage inhibition data were statistically analyzed using the Genstat computer package (VSN International, 2008) and the means were separated by the least significant differences (LSD).

3.3.4.2 Microtitre dilution method

Malt extract broth was inoculated with *Colletotrichum* and incubated for 5 d at 25°C. The

broth was adjusted to 0.2 optical density (thus 1 x 10^5 and 1 x 10^6 spores.ml^{-1} for *C. lindemuthianum* and *C. dematium*, respectively) using an electrospectrometer just before inoculation.

Fifty milligrams of each of the plant extracts were dissolved in 1000 µl of 10 % DMSO in order to yield a series of 50 mg.ml^{-1} solutions of extracts. Ninety-six well microtitre plates were used and 100 µl of the broth was added to all the wells. To the first three wells of row A, 100 µl of stock solution (50 mg.ml^{-1}) of acetone plant extracts were added, in the 4th to 6th wells ethyl acetate plant extracts and to wells 7 to 9 water extracts were added. To well 10, nutrient broth was added as a negative control. Celest® XL (100 ml of 2.5 ml.ml^{-1} (v/v) was added in well 11 as a positive control. To well 12, 100 µl of 10 % DMSO was added. A series of dilutions were carried out to row H.

Six-day-old malt broth cultures (100 µl) of *Colletotrichum* fungi were added to each well. The plates were divided into two sets; the first set (six plates) was inoculated with *C. lindemuthianum* and the other with *C. dematium*. The plates were then covered with lids, sealed with Parafilm® and incubated at ±25 ^{o}C for 48 h. Thereafter, 40 µl of 0.2 mg.ml^{-1} iodinitrotetrazolium chloride (INT) was added to all the wells, with the exception of columns 3, 6 and 9, as a growth indicator to determine the minimum inhibitory concentration (MIC) values for the 18 plant extracts. The microtitre plates were incubated for 24 h and evaluated. The last clear wells were regarded as the MICs of extracts. The experiment was repeated three times and the final MIC for the extracts was calculated as described by Fawole *et al.* (2009). A schematic diagram of experimental design of the 96-well microtitre plate which was used in the study is shown in Figure 3.1.

3.4 RESULTS

3.4.1 Agar infusion technique

Figure 3.2A shows the inhibitory effect of acetone plant extracts on *C. lindemuthianum* at 6 DAI. *Allium* acetone extracts completely (100 %) inhibited *C. lindemuthianum* fungi at all the concentrations and *Agapanthus* acetone extracts completely (100 %) inhibited *C. lindemuthianum* at 5.0 mg.ml^{-1}. The inhibition increased as the concentrations of all extracts increased with the exception of *Chlorophytum* and *Ipomoea* leaf extracts. In fact, *Chlorophytum* extracts stimulated fungal growth as the concentration increased. *Allium* acetone extracts completely (100 %) inhibited the growth of *C. dematium* at 5.0 mg.ml^{-1} only where as *Carica* and *Agapanthus* acetone extracts inhibited the growth of *C. dematium* by 96 % and 90 %, respectively, at the highest concentration (Fig. 3.2B). Likewise in *C. lindemuthianum*, the increase in *Chlorophytum* and *Ipomoea* acetone extracts concentrations promoted the growth of the *C. dematium*.

Allium ethyl acetate extracts completely inhibited (100 %) the growth of *C. lindemuthianum* at 2.5 and 5.0 mg.ml^{-1}, respectively (Fig. 3.3A). *Syzygium* ethyl acetate extract inhibited the growth of *C. lindemuthianum* by 80 % at 5.0 mg.ml^{-1}. All other ethyl acetate plant extracts (*Agapanthus*, *Carica*, *Chlorophytum* and *Ipomoea*) failed to inhibit *C. lindemuthianum* by 50 % and above at all concentrations. *Allium* ethyl acetate extract inhibited the growth of *C. dematium* by 87 % but the other plant ethyl acetate extracts inhibited the growth of *C. dematium* by less than 50 % (Fig. 3.3B). *Ipomoea* ethyl acetate extracts promoted the growth of *C. lindemuthianum* and *C. dematium* as the concentrations increased.

Carica water extract completely (100 %) inhibited the growth of *C. lindemuthianum* at 5.0 mg.ml^{-1} followed by *Chlorophytum* and *Agapanthus* water extracts that inhibited the growth by 95 % and 89 %, respectively (Fig. 3.4A). *Agapanthus* water extracts inhibited the

growth of *C. dematium* by 80 % whereas the rest of the water extracts failed to reach 50 % inhibition (Fig. 3.4B). *Ipomoea* water extracts recorded negative inhibition values at all the concentrations on *C. dematium*. The *Chlorophytum* water extracts promoted the growth of *C. dematium*.

Table 3.2 shows the mean inhibition and inhibition percentage of plant extracts at 5.0 $mg.ml^{-1}$ and the control treatments on *C. lindemuthianum* after 3, 6 and 9 DIA. All the extracts were highly significant ($P \leq 0.001$). *Agapanthus* and *Allium* acetone extracts completely (100 %) inhibited *C. lindemuthianum* after 9 DAI. The two extracts were better than the synthetic fungicide, Celest® XL. *Syzygium* acetone extract was another extract that performed better than Celest® XL on the 9th DAI, though its performance was similar to the latter at 3 and 6 DAI. *Chlorophytum* acetone extracts, ethyl acetate and acetone (the negative controls) were the poorest performers such that they promoted the growth of *C. lindemuthianum*. Only *Allium* ethyl acetate extract showed a high rate of inhibition (96.4 and 100 %) of *C. lindemuthianum* at 3, 6 and 9 DAI similar to that of Celest® XL. All other ethyl acetate plant extracts promoted growth of *C. lindemuthianum* except *Agapanthus* and *Syzygium* extracts.

Carica, *Agapanthus* and *Chlorophytum* water extracts had the highest inhibition percentages of *C. lindemuthianum* and their performance was similar to that of Celest® XL. *Carica* water extracts had the highest (100 %) inhibition at 9 DAI. There was a decrease in inhibition by extracts as DAI increased in most cases.

All plants extracts were significantly ($P \leq 0.001$) different in their inhibition of the growth of *C. dematium* (Table 3.3). *Allium* acetone extracts showed complete (100 %) inhibition at 3, 6, and 9 DAI. *Carica*, *Agapanthus* and Celest® XL showed complete (100 %) inhibition at 3 DAI but their inhibition percentage declined with the increase in DAI. The inhibition of *Carica* acetone extract and Celest® XL were not significant at 6 and 9 DAI but

Celest® XL was significantly (P≥0.001) different to *Agapanthus* acetone extracts in inhibiting *C. dematium* on 6 and 9 DAI.

No ethyl acetate extract showed a complete (100 %) inhibition of the growth of *C. dematium*. All ethyl acetate extracts had an inhibition percentage of less than 50 % except the *Allium* extract that recorded 62.2 and 71.8 %, respectively, as its lowest and the highest means inhibition percentage, respectively.

The water extracts did not completely (100 %) inhibit the growth of *C. dematium* (Table 3.3) but though that was the case, *Agapanthus* performed as well as Celest® XL at 9 DAI. The inhibition percentage of *Agapanthus* water extracts on *C. dematium* increased with increase in DAI but the inhibition of all other extracts and Celest® XL decreased with increase in DAI.

3.4.2 Microtitre dilution method

Table 3.4 shows the MIC values of different plant extracts extracted with acetone, ethyl acetate and water. All the *Syzygium* extracts were active against *C. dematium* and *C. lindemuthianum*. *Allium* extracts were also active against *C. dematium* and *C. lindemuthianum*, however, the water extract failed to inhibit the growth of *C. lindemuthianum*. *Agapanthus* acetone and ethyl acetate extracts were active against both fungi with MICs of between 3.13 to 6.25 $mg.ml^{-1}$ and 1.56 to 3.13 $mg.ml^{-1}$ respectively, but the water extracts failed to produce a MIC. *Carica* and *Chlorophytum* water extracts were active against *C. lindemuthianum* but their organic extracts were inactive against both *C. dematium* and *C. lindemuthianum*. All the *Ipomoea* extracts and DMSO were inactive against both *C. dematium* and *C. lindemuthianum*. Celest® XL showed superior inhibitory activity when compared to all the plant extracts.

3.5 DISCUSSION

Allium acetone and ethyl acetate extracts compared well with Celest® XL by completely (100 %) inhibiting *C. lindemuthianum* and *C. dematium* in the agar infusion technique. The *Allium* water extracts inhibited the growth of *C. dematium* in the microtitre dilution technique. The performance of *Allium* water extracts on *C. dematium* is similar to the findings of Shovan *et al.* (2008) where *Allium* water extracts inhibited the growth of *C. dematium*.

Chlorophytum water extracts had higher inhibition values on *C. lindemuthianum* than acetone or ethyl acetate and it can be speculated that the *C. lindemuthianum* antifungal chemical in *Chlorophytum* is only soluble in water and not in acetone and ethyl acetate. *Chlorophytum* water extracts failed to inhibit the growth of *C. dematium* which contrasted with the findings of Raghavenda and colleagues (2006) where aqueous extracts of *Chlorophytum boriviliam* reduced the incidence of *C. dematium*. The variation in results can be due to differences in species of *Chlorophytum* used in the two studies.

Carica leaf water extracts at 5.0 $mg.ml^{-1}$ performed well and was comparable to Celest® XL, by completely (100 %) inhibiting the growth of *C. lindemuthianum* in the agar infusion technique and had a MIC of 1.56 $mg.ml^{-1}$ but failed to inhibit the growth of *C. dematium*. The fungal toxicity of *Carica* leaf water extracts against the powdery mildew plant pathogen was reported by Amadioha (1998). The failure of *Carica* water extracts to inhibit the growth of *C. dematium* could be due to differences in chemical or genetic composition between the two *Colletotrichum* spp. used in this study. The performance of *Carica* water extracts on *C. lindemuthianum* was superior over that of organic extracts in both techniques used in this study and these results are in agreement with the findings of Anibijuwon and Udeze (2009) who reported that *Carica* leaf water extracts gave higher inhibition against bacteria than organic extracts.

All *Syzygium* extracts were active against both *C. dematium* and *C. lindemuthianum* in the microtitre dilution experiment and the results were similar to those obtained in the agar infusion experiment even though it did not completely inhibit the growth of the two fungi. There was, however, differences between the results of the two experiments as far as the performance of ethyl acetate and water extracts against *C. dematium* were concerned.

The growth of fungi increased with increased concentration of *Ipomoea* leaf extracts. It can therefore be speculated that *Ipomoea* leaves have some constituents that are needed by the *Colletotrichum* fungi for growth hence their rapid vegetative growth.

Generally, there was an increase in inhibition of fungal growth by plant extracts as concentrations increased and this could have been due to the increased availability of the antifungal chemicals in the media.

Acetone plant extracts showed the highest inhibitory activity among the other extracts. Similar results pertaining to the higher inhibition of acetone extracts were reported by Masoko *et al.* (2005) who stated that acetone extracts were superior to other extraction solvents such as hexane, dichloromethane and methanol. The sequential extracting method could have a contribution towards the poor performance of ethyl acetate extracts compared to acetone extracts because most of the organic soluble chemicals in plants had already be extracted in the acetone and little remained in the residues. The water extracts gave the poorest results in this study as speculated by Masoko *et al*. (2005) who stated that water fails to extract non-polar active compounds in plant materials.

Allium, *Agapanthus*, *Carica*, and *Syzygium* acetone extracts showed antifungal activities against both *C. lindemuthianum* and *C. dematium*. *Allium* ethyl acetate also showed antifungal activity against both *Colletotrichum* species whereas *Syzygium* ethyl acetate only showed antifungal activity against *C. lindemuthianum*. *Agapanthus* water extracts exhibited antifungal activity against both fungi whereas *Carica*, *Chlorophytum* and *Syzygium* water

extracts were toxic to *C. lindemuthianum*. *Ipomoea* extracts promoted the growth of *Colletotrichum* fungi. *Colletotrichum lindemuthianum* succumbed easier to extracts than *C. dematium*.

This study has contributed an additional list of plants that can potentially be used by smallholder farmers to control anthracnose diseases of common bean and cowpea. There is, however, a need to further investigate the performance of water and acetone *Allium*, *Agapanthus*, *Carica*, *Syzygium* and *Chlorophytum* extracts *in vivo* as seed treatments and as a spray to control anthracnose disease of cowpea and common bean.

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	1	2	3	4	5	6	7	8	9	10	11	12
	Acetone extracts			Ethyl acetate extracts			Water extracts			Nutrient Broth	Fungicide Celest XL	DMSO
A	12.5 mg/ml											
B	6.25 mg/ml											
C	3.13 mg/ml											
D	1.56 mg/ml											
E	0.78 mg/ml											
F	0.39 mg/ml											
G	0.19 mg/ml											
H	0.095 mg/ml											

Figure 3.1: Schematic representation of the 96 microtitre plate dilutions used in the study.

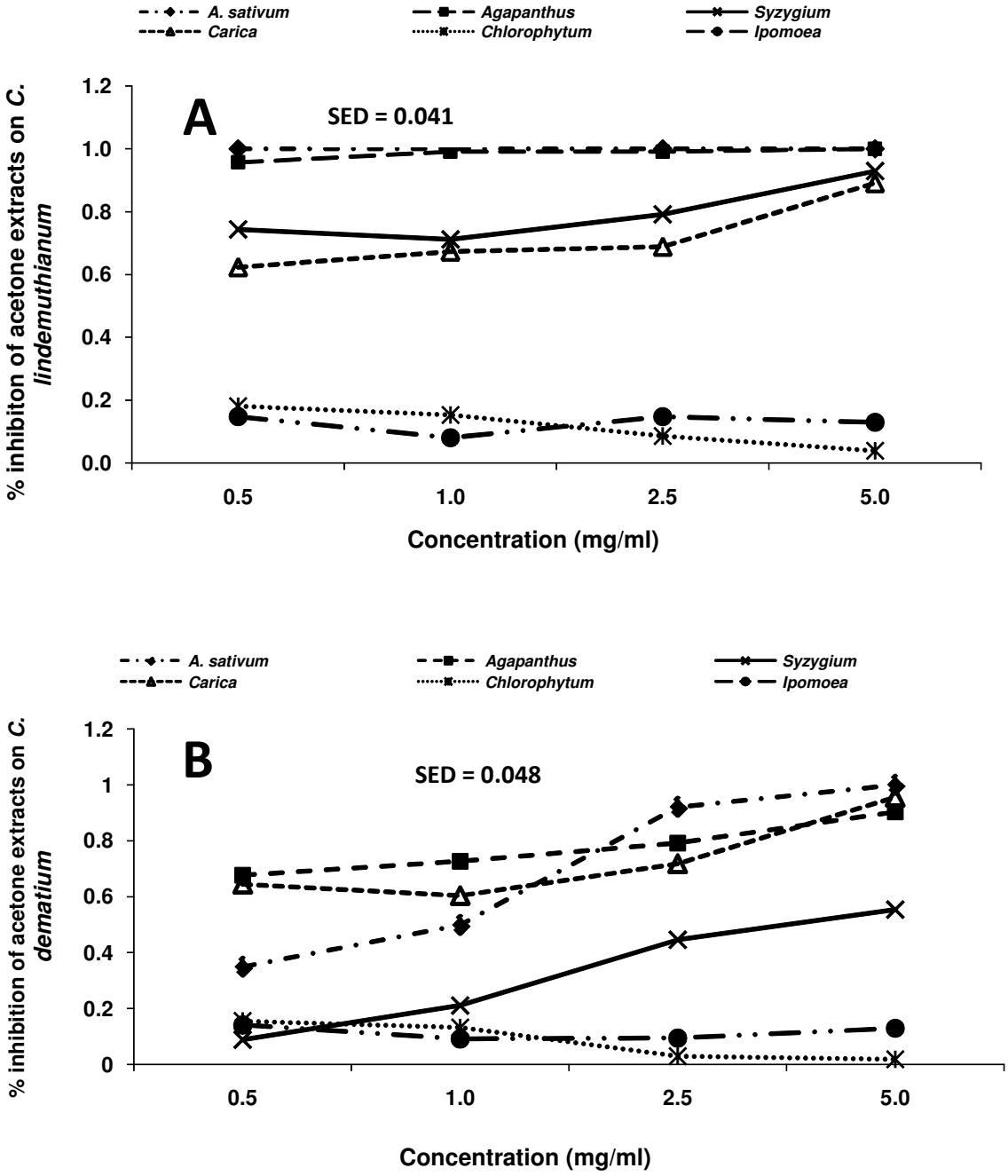


Figure 3.2: The effect of acetone crude plant extracts on the colony growth of *C. lindemuthianum* (A) and *C. dematium* (B) at six days after inoculation.

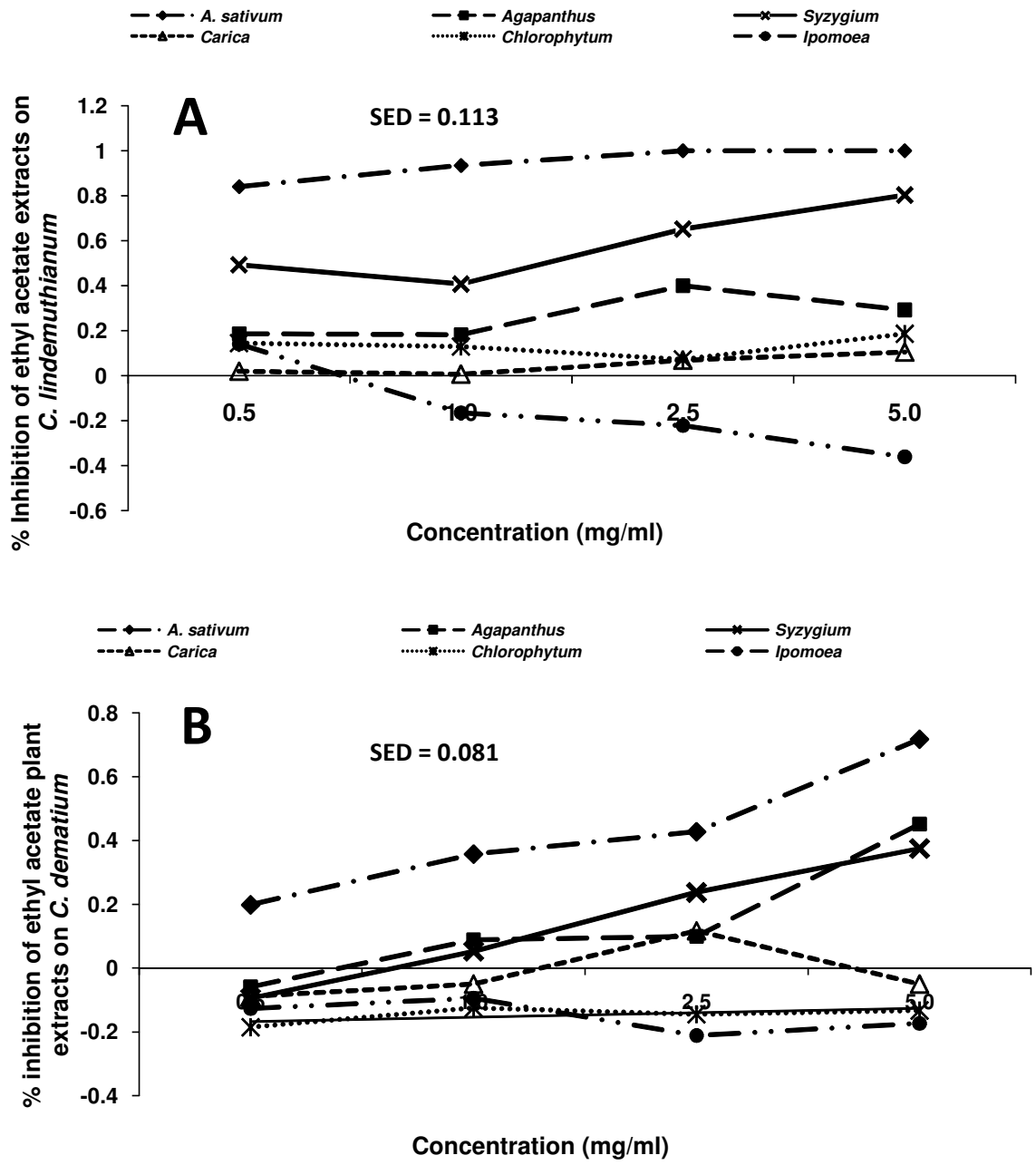
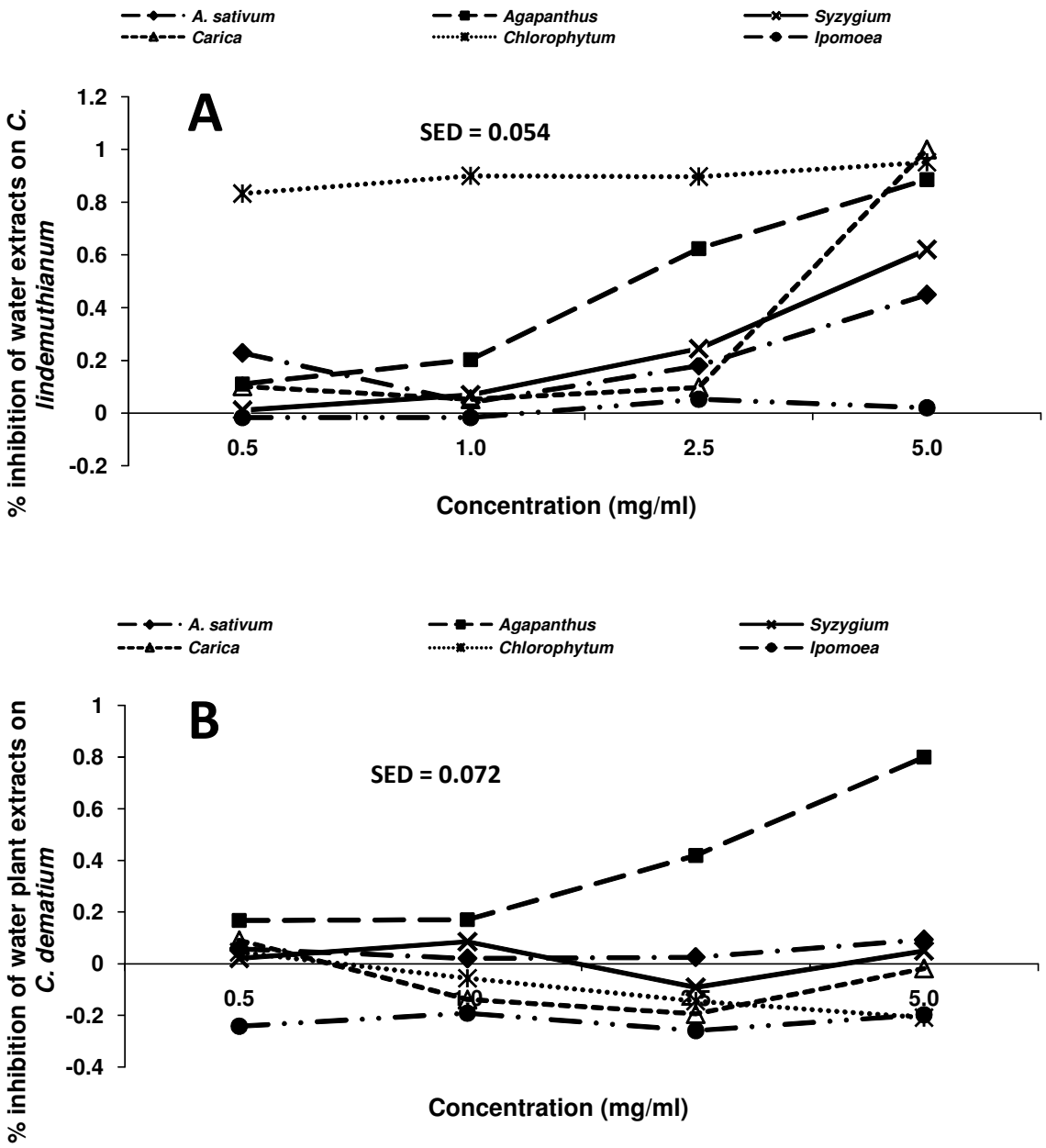


Figure 3.3: The effect of ethyl acetate crude plant extracts on the colony growth of *C. lindemuthianum* (A) and *C. dematium* (B) at six days after inoculation.



Figures 3.4: The effect of water crude plant extracts on the colony growth of *C. lindemuthianum* (A) and *C. dematium* (B) at six days after inoculation.

Table 3.1: The quantities of crude extracts in grams harvested from 1 kg plant material using acetone, ethyl acetate and water as solvents.

Plant name	Voucher number	Solvent		
		Acetone	Ethyl acetate	Water
Agapanthus caulescens	PRU96528*	7	12.94	52.53
Allium sativum	PRU96551*	13.6	3.86	413.94
Carica papaya	PRU96552*	32.86	15.05	103.11
Chlorophytum comosum	PRU96518*	nd	nd	nd
Ipomoea batatas	PRU96529*	nd	nd	nd
Syzygium cordatum	PRU96517*	19.7	18.2	125.3

nd = not determined; * voucher number assigned by the University of Pretoria H.G.W.J. Schweickerdt Herbarium.

Table 3.2: Inhibition of plant extracts on *Colletotrichum lindemuthianum* as compared to the controls at 3, 6 and 9 DAI.

Extract (5.0 mg.ml^{-1})	Solvent								
	Acetone			Ethyl acetate			Water		
	3 days	6 days	9 days	3 days	6 days	9 days	3 days	6 days	9 days
Carica papaya	0.0 a (100)	2.88 b (89.1)	12.5 c (67.5)	11.12 bc (-5.9)	27.38 d (-3.8)	44.5 e (-15.9)	0.0 a (100)	0.0 a (100)	0.0 a (100)
Agapanthus caulescens	0.0 a (100)	0.0 a (100)	0.0 a (100)	10.12 b (3.6)	21.62 c (18.0)	31.38 c (18.2)	0.0 a (100)	3.5 a (88.6)	5.5 b (85.7)
Chlorophytum comosum	9.62 bc (8.4)	25.38 d (3.9)	40.0 f (-4.2)	12.0 c (-5.9)	24.9 cd (5.7)	41.0 de (-6.8)	1.0 a (93.9)	1.5 a (95.1)	5.9 b (84.7)
Syzygium cordatum	0.0 a (100)	1.88 ab (92.9)	6.12 b (84.1)	0.75 a (92.9)	6.0 b (77.3)	14.25 b (62.9)	1.12 a (93)	11.62 b (62)	30.4 d (20.8)
Allium sativum	0.0 a (100)	0.0 a (100)	0.0 a (100)	0.0 a (100)	0.0 a (100)	1.38 a (96.4)	5.5 b (66.3)	11.88 b (55.0)	24.5 c (36.2)
Ipomoea batatas	8.62 b (17.9)	22.88 c (13)	34.12 de (11.1)	13.6 d (-29.7)	26.38 e (-100)	41.62 de (-8.4)	12.88 d (21)	30.0 d (-13.7)	30.0 c (6.2)
Celest®XL	0.0 a (100)	0.25 ab (99.1)	9.12 c (76.24)	0.0 a (100)	0.25 a (99.1)	9.12 b (76.24)	0.0 a (100)	0.25 a (99.1)	9.12 b (76.24)
Acetone	11.62 c (-10.7)	26.5 d (-0.45)	40.38 f (-5.21)	11.62 bc (-10.7)	26.5 d (-0.45)	40.38 de (-5.21)	11.62 cd (-10.7)	26.5 cd (-0.45)	40.38 e (-5.21)
Ethyl acetate	15.75 d (-50.0)	26.62 d (-0.9)	38.62 f (-0.63)	15.75 e (-50.0)	26.62 d (-0.9)	38.62 d (-0.63)	15.75 e (-50.0)	26.62 cd (-0.9)	38.62 e (-0.63)
PDA only	10.5 c	26.38 d	38.38 ef	10.5 bc	26.38 d	38.38 d	10.5 c	26.38 c	38.38 e
P≤0.001	***	***	***	***	***	***	***	***	***
LSD	1.16	1.94	4.28	1.85	3.81	5.44	1.74	3.52	4.19

The values bearing same letters are not significantly different (P≤0.001).
Values in brackets indicate percentage inhibition of extracts and values with letters are mean fungal growth values.

Table 3.3: Inhibition of plant extracts on *Colletotrichum dematium* as compared to the controls at 3, 6 and 9 DAI.

Extract (5.0 mg.ml^{-1})	Solvent								
	Acetone			Ethyl acetate			Water		
	3 days	6 days	9 days	3 days	6 days	9 days	3 days	6 days	9 days
Carica papaya	0.0 a (100)	1.88 ab (95.6)	11.5 bc (79.1)	24.12 e (11.6)	44.75 de (-5)	55.0 d (0.0)	11.88 c (56.2)	43.38 cd (-1.8)	55.0 b (0.0)
Agapanthus caulescens	0.0 a (100)	4.12 b (90.3)	15.0 c (72.7)	15.0 c (44.7)	23.38 c (45.2)	49.25 d (10.0)	7.38 b (72.8)	8.50 b (80)	10.0 a (81.8)
Chlorophytum comosum	19.0 c (30)	41.88 e (1.8)	55 e (0.0)	25.5 e (5.99)	48.25 de (-13.2)	54.4 d (0.9)	22.75 ed (16.1)	51.5 f (-20.8)	55.0 b (0.0)
Syzygium cordatum	2.0 ab (92.6)	19.0 c (55.4)	35.88 d (34.8)	14.5 c (46.5)	26.62 de (37.5)	39.5 c (24.4)	19.0 d (30)	40.5 c (5.0)	55.0 b (0.0)
Allium sativum	(0.0 a (100)	0.0 a (100)	0.0 a (100)	10.25 b (62.2)	12.0 b (71.8)	17.25 b (68.6)	13.88 c (48.8)	38.62 c (9.4)	55.0 b (0.0)
Ipomoea batatas	19.6 c (28.11)	37.12 d (12.9)	55.0 e (0.0)	25.25 e (6.9)	50.0 e (-17.3)	55.0 d (0.0)	27 f (-0.3)	51.0 ef (-19.7)	55.0 b (0.0)
Celest®XL	0.0 a (100)	0.25 a (99.4)	9.12 b (83.4)	0.0 a (100)	0.25 a (99.4)	9.12 a (83.4)	0.0 a (100)	0.25 a (99.4)	9.12 a (83.4)
Acetone	20.5 c (24.4)	43.62 c (-2.35)	55.0 e (0.0)	20.5 d (24.4)	43.62 de (-2.35)	55.0 d (0)	20.5 d (24.4)	43.62 cde (-2.35)	55.0 b (0.0)
Ethyl acetate	25.0 d (7.82)	41.5 c (2.63)	54.5 e (1.0)	25.0 e (7.82)	41.5 d (2.63)	54.5 d (1.0)	25.0 ef (7.82)	49.5 def (2.63)	54.5 b (1.0)
PDA only	27.12 d	42.62 e	55.0 e	27.12 e	42.62 d	55.0 d	27.12 f	42.62 cd	55.0 b
P≤0.001	***	***	***	***	***	***	***	***	***
LSD	2.4	3.72	5.14	3.33	7.14	5.86	3.81	7.53	3.75

The values bearing same letters are not significantly different (P≤0.001).
Values in brackets indicate percentage inhibition of extracts and values with letters are mean fungal growth values.

Table 3.4: The minimum inhibitory concentration (MIC) of selected plants extracts on *Colletotrichum dematium* and *Colletotrichum lindemuthianum.*

	SOLVENT					
	ACETONE		ETHYL ACETATE		WATER	
PLANT EXTRACTS	CL ($mg.ml^{-1}$)	CD ($mg.ml^{-1}$)	CL ($mg.ml^{-1}$)	CD ($mg.ml^{-1}$)	CL ($mg.ml^{-1}$)	CD ($mg.ml^{-1}$)
Allium sativum	0.78	0.78	0.78	3.13	0	6.25
Agapanthus caulescens	6.25	3.13	3.13	1.56	0	0
Syzygium cordatum	3.13	6.25	0.78	1.56	1.56	3.13
Carica papaya	0	0	0	0	1.56	0
Chlorophytum comosum	0	0	0	0	12.5	12.5
Ipomoea batatas	0	0	0	0	0	0
Celest® XL	0.09	0.09	0.09	0.09	0.09	0.09
DMSO	0	0	0	0	0	0

CL = *Colletotrichum lindemuthianum*; CD = *Colletotrichum dematium.*

CHAPTER FOUR

THE EFFECT OF CRUDE PLANT EXTRACTS ON SEED GERMINATION, EMERGENCE AND GROWTH OF COMMON BEAN (*PHASEOLUS VULGARIS* L.) AND COWPEA (*VIGNA UNGUICULATA* L. WALP) AND THE CONTROL OF ANTHRACNOSE DISEASE

4.1 ABSTRACT

The study was initiated to evaluate the effects of plant extracts as a seed treatment on cowpea (*Vigna unguiculata* L. Walp) and common bean (*Phaseolus vulgaris* L.) seed germination, emergence, growth and the control of anthracnose disease caused by *Colletotrichum dematium* (Fr.) Grove var. *truncata* and *Colletotrichum lindemuthianum* (Sacc. and Magn.) Bri. and Cav. The crude water and acetone extracts of *Agapanthus caulescens* Spreng., *Allium sativum* L., *Carica papaya* L. and *Syzygium cordatum* Hochst .ex Krauss were tested at 5 and 15 mg.ml^{-1}. Celest® XL and non-inoculated seeds represented the positive controls whereas DMSO and inoculated seeds represented the negative controls. The low concentrations (5 mg.ml^{-1}) of *Syzygium* and *Agapanthus* water extracts and acetone extracts of *Agapanthus* and *Carica* gave a high percentage bean seed germination, emergence, a short mean emergence time (MET) and were effective in controlling anthracnose. *Agapanthus* (both water and acetone) extracts also increased the shoot length and dry weight of the seedlings. The *Allium* acetone extract (5 mg.ml^{-1}) was the only treatment that gave good results with respect to germination percentages, MET, shoot length, leaf area and dry mass of cowpea. Five mg.ml^{-1} concentrations of *Syzygium* and *Agapanthus* water extracts and acetone extracts of *Agapanthus* and *Carica* have potential as seed treatments on bean. *Allium* acetone extract (5 mg.ml^{-1}) was the only potential cowpea seed

treatment that could be recommended to farmers as an alternative to synthetic fungicides.

Key words: anthracnose, germination, *Phaseolus* v*ulgaris*, plant extracts, seed emergence, *Vigna unguiculata*.

4.2. INTRODUCTION

Africa is the largest cowpea (*Vigna unguiculata* L. Walp) producer (Pereira *et al*., 2001) and second largest common bean (*Phaseolus vulgaris* L.) producer (FAO, 2010). The two crops are a cheap source of protein for most rural people on the continent. Cowpea and common bean have the ability of fixing atmospheric nitrogen into the soil (Summerfield *et al*., 1977; Kolawale *et al*., 2000; Singinga *et al.,* 2000; Olivera *et al*., 2004; Rondon *et al.*, 2006;) hence, are occasionally grown as soil improvers and as animal feed (Purseglove, 1968; Quin, 1997).

There are many diseases that infect cowpea and common bean and anthracnose disease is one of the most destructive seed-borne fungal diseases of both crops in the sub-Sahara region. The disease is prevalent in smallholder farmers' fields due minimal fungicide use (Allen, 1995). This disease is caused by pathogens of the genus *Colletotrichum*. Bean anthracnose is caused by *Colletotrichum lindemuthianum* (Sacc. and Magn.) Bri. and Cav. whereas cowpea anthracnose is caused by *Colletotrichum dematium* (Fr.) Grove var. *truncata* (Pakela *et al.*, 2002; Smith and Aveling, 1997) and *C. lindemuthianum* (Williams, 1975; Allen *et al.*, 1996). According to Allen *et al.* (1996), total yield losses of bean and 50 % loss in cowpea (Emechebe and Florini, 1997) can occur in cases where infected seeds have been planted. There are many ways of managing anthracnose disease such as use of disease free seed, planting of anthracnose resistant varieties and use of chemicals. The use of resistant varieties has failed the farmers due to the presence of several races of *Colletotrichum*

(Dillard, 1988). The behaviour of most African smallholder farmers to exchange seeds has contributed to the failure of resistant varieties because this act introduced new races of *Colletotrichum* in areas where they never existed before (Dron and Bailey, 1995).

Chemical seed treatment is the only form of crop insurance that protects the seed from pest and disease attack (Bierman *et al.*, 2006; Gwary *et al.,* 2007). It is a cheaper and effective way of controlling seed-borne plant diseases (Kreitlow*et al.,* 2010). Seed treatment kills pathogens carried within and on the seed surface. It also protects seed or seedlings from soil pathogens and reduces the amount of pesticides required to control the disease (Anonymous, 1992) thereby reducing environmental contamination.

The synthetic chemical fungicides that were reported to be effective against anthracnose disease as seed treatments are captan-thiram-metalaxyl-benomyl (CTMB), diazinon-captan-thiophanate methyl (DCT), thiram-metalaxyl-thiabendazole (TMZ) and carbathiin - thiram (Anchor) (Tu and Zheng, 1994). Cowpea and common bean are rarely treated before sowing because synthetic fungicides are rarely accessible to the poor resource smallholder farmers in developing countries due to their price. Therefore, cheap, environmentally and human friendly fungicidal compounds are needed to be used as seed treatments for smallholder farmers.

The extracts of garlic (*Allium sativum* L.), neem (*Azadirachta indica* A. Juss), pawpaw leaf (*Carica papaya* L.), *Moringa oleifera* Lam., bitter leaf (*Vernonia amygdalina* L.) and *Syzygium cumini* L. Skeels have been tested at different concentrations against other seed-borne and foliar diseases of African yam bean (*Sphenostylis stenocarpa* (Hochst ex. Rich) Harms), cowpea, jute (*Corchorus capsularis* L.), chilli (*Capsicum annum* L.) and jowar (*Sorghum bicolor* (L.) Moench.) by seed soaking for different lengths of time and good results have been reported (Islam *et al*., 2001; Nwachukwu and Umechuruba, 2001; Somda *et al*., 2007; Ogwulumba *et al.,* 2008). Some plant extracts namely *Bryophyllum pinnantus*

Kurz, caraway (*Carum carvi* L.), *Eucalyptus globules* (Caliptos) Labill, *Ocimum gratissimum* Closium L., peppermint (*Mentha piperita* L.), *Syzygium cumini* and *Vernonia amygdalina* effectively control diseases without any negative side effects on germination and growth of African yam bean, cowpea, pea (*Pisum sativum* L.) and wheat (*Triticum aestivum* L.) (Nwachukwu and Umechuruba, 2001; Alabi *et al.*, 2005; Shafique *et al.,* 2007; El-Mougy and Alhabeb, 2009).

It is against the above background that this study was initiated to evaluate the efficacy of plant extracts as a seed treatment against anthracnose disease of cowpea and bean. This study also aimed at investigating the effect the plant extracts had on seed germination, emergence and growth of cowpea and common bean.

4.3 MATERIALS AND METHODS

4.3.1 Experimental site

The experiments were conducted in the Govender Seed laboratory and the greenhouses of the Department of Microbiology and Plant Pathology, University of Pretoria.

4.3.2 Collection of plants and preparation of crude extracts

The sites of plant collection and the methodology for the preparation crude extracts were the same as indicated in Chapter 3. In this study, however, no ethyl acetate extracts were used. The acetone and water crude extracts of *Agapanthus caulescens* Spreng. (whole plant), *Syzygium cordatum* Hochst.ex C. Krauss(fruits), *Allium sativum* (bulb) and *Carica papaya* (leaves) were used and their selection was based on the results obtained in previous experiments (Chapter 3). The sites of plant collections and crude extract preparation were the same as indicated in Chapter 3.

4.3.3 Origin of common bean and cowpea seeds

Common bean (Jenny variety) seeds from 2009 and 2010 production seasons were obtained from Pan Farm in Middelburg, Mpumalanga. Cowpea, IT93K5132 variety (cream coloured) and Pan 311 variety (brown coloured), seeds used in this study were collected from the Department of Agriculture, Nelspruit, Mpumalanga.

4.3.4 Extracts and chemical seed treatment

Seeds were washed with tap water in a clean plastic bowl prior to treatment with either extracts or chemical. Water extracts were dissolved directly in sterile distilled water and the acetone crude extracts were dissolved in 1 % dimethyl sulfoxide (DMSO) sterile distilled water. Both extracts were dissolved to yield final concentrations of 5 and 15 $mg.ml^{-1}$. Seeds were soaked in the extracts and placed in the dark for 24 h at 24±1 ^{o}C. For the negative controls, seeds were soaked in sterile distilled water or 1 % DMSO, dissolved in sterile distilled water, for 24 h in the dark at 24±1 ^{o}C. The positive control seeds were treated with Celest® XL (25 gai/L) (fludioxonil and mefenoxam, a synthetic fungicide from Syngenta SA, Midrand). The seeds were then left to air dry in a laminar flow bench for 1 h.

4.3.5 Standard germination test

The germination test was conducted according to a modified method of the procedure used by the International Seed Testing Association (ISTA) (ISTA, 2011) using rolled paper towels. Each treatment was replicated four times with 50 seeds per replicate. The sheets were moistened with 120 ml sterile distilled water. Each roll consisted of two sheets of germination paper, followed by a layer of white paper towel and then a third sheet of germination paper. Fifty seeds were linearly placed on the third sheet and the fourth sheet of germination paper was used to cover the seeds. The germination paper towels were rolled and

placed in labeled polyethelene bags and sealed with rubber bands. The polythelene bags were put in an upright position and then incubated at room temperature (24 ± 2 ^{o}C) and 12 h light. First rating for cowpea and bean seed germination was four and five days after incubation, respectively. The seeds were then incubated for a further five days after which the number of normal, abnormal seedlings and dead seeds was recorded according to the ISTA rules (ISTA, 2011). The abnormal seedlings and dead seeds data were square root transformed before analysis but the normal seedlings and the germination percentage data were analysed untransformed using the Genstat computer package (VSNi, 2008). The experiment was repeated twice.

4.3.6. Greenhouse experiments

Cowpea and bean seed were surface sterilized using 1% sodium hypochloride for five minutes and rinsed thrice in sterile distilled water. The sterilized seeds were then soaked in sterile distilled water for 30 min at room temperature (24±1 ^{o}C). A small hole was made on one cotyledon of each seed through the seed coat with a sterilized needle before being inoculated with the individual *Colletotrichum* spp. by soaking seeds in a 3.7 x 10^{-5} spore suspension for 4 h in the dark at 24±2 ^{o}C. Bean seeds were inoculated with *C. lindemuthianum* and cowpea with *C. dematium*. The inoculated seeds were placed in a high humidity condition by covering the mouth of flasks in which seeds were with polyethene and sealing with rubber bands. The flasks were placed in the dark for 16 h at 24±2 ^{o}C in order for the fungi to infect them. Thereafter the seeds were air dried in a laminar flow bench before being treated with the acetone and water plant extracts. Inoculated seeds were treated with plant extract solutions by soaking them in 5 and 15 mg.ml^{-1} concentrations for 24 h in the dark at 24±2 ^{o}C. Inoculated seeds treated with Celest® XL and non-inoculated seed soaked in water were the positive controls. The negative controls were inoculated seeds soaked in water

or 1% DMSO. Both the positive and negative controls were subjected to the same light and temperature conditions as the seeds treated with the plant extracts.

The seeds were then sown in 15 cm (D) x 12.5 cm (H) pots filled with a steam sterilized mixture of sand and garden compost (Culterra (Pty) Ltd, Muldersdrif) in a ratio of 1:1 (m/m). Sterilization of sand and compost mixture was done twice at 99 ^{o}C for 3 h in an Electrode Steam Boiler (Marshall-Fowler, Randfontein). Each pot was seeded with six seeds and each treatment was replicated four times and placed in a randomized block design. The moisture was maintained by irrigating daily (RH was not determined) and 14 and 27 ^{o}C were the minimum and maximum temperatures within the greenhouse.

The data collected included the number of emerged seedlings per plot per day, shoot length (mm), wet mass (g), dry mass (g), leaf area (cm^2) and the incidence and severity of anthracnose disease. Number of the emerged seedlings per day per plot was collected by counting the emerged seedlings daily at cotyledon appearance. Seedling emergence counts were terminated on day 12 after sowing when no further emergence was recorded.

Mean emergence time (MET) was calculated as $\text{MET} = \frac{\sum(n \times g)}{N}$,

where n is the number of seedlings emerging per day, g is the number of days needed for emergence, and N is the total number of emerged seeds (Benvenuti *et al.,* 2001).

Shoot length was recorded weekly and disease incidence and severity were recorded every fortnight. The wet mass, dry mass and leaf area were determined after harvest. The disease incidence was calculated by counting the number of plants showing anthracnose symptoms and expressed as a percentage total of the total number of plants per plot.

$$\text{DI} = \frac{No\ of\ infected\ \frac{\text{plants}}{\text{plot}}}{Total\ No.\ of\ \frac{\text{plants}}{\text{plot}}}\ x100$$

The anthracnose disease severity scale of 0 – 9 (CIAT, 1987) was employed where:

1 : no visible disease symptoms.

3 : presence of very few and small lesions, mostly on the primary vein on the lower leaf surface.

5 : presence of several small lesions on the petiole or on the primary and secondary veins of the lower leaf.

7 : presence of numerous enlarged lesions on the lower side of the leaf. Necrotic lesions can be observed on the upper leaf surface and on the petioles.

9 : severe necrosis on 25% or more of the plant tissues are evident as a result of lesions on the leaves, petioles, stems, branches, and even on the growing point, which often results in death of the plant tissues.

The disease severity scores of 1 to 3 were regarded as effective where as those of 4 to 9 not effective in controlling the anthracnose disease in this study. Data was analyzed by using the Genstat Discovery 3 statistical package (VSNi, 2008).

4.4 RESULTS

4.4.1 Cowpea germination, emergence and mean emergence time

Differences in the germination percentage and percentage normal seedlings showed significant differences ($P \leq 0.05$) in the IT93K5132 cowpea seeds and insignificant differences in PAN 311 seeds (Table 4.1). The IT93K5132 seeds treated with water extracts of *Allium* (5 and 15 $mg.ml^{-1}$), 5 $mg.ml^{-1}$ *Agapanthus*, 5 and 15 $mg.ml^{-1}$ *Carica*, 5 $mg.ml^{-1}$ *Syzygium* and acetone extracts of *Allium*, *Agapanthus*, 5 $mg.ml^{-1}$ *Syzygium* and Celest® XL showed a significantly ($P \leq 0.05$) higher cowpea germination percentage than the negative controls and other plant extracts tested in this study. All the plant extracts showed higher percentage normal seedlings and were not significant ($P \leq 0.05$) from each other except for the higher *Syzygium* acetone extract and the negative controls.

The IT93K5232 and PAN 311 cowpea seedlings emergence percentages were significant (P≤0.05) between treatments (Table 4.3). The 5 mg.ml^{-1} *Allium* acetone, 5 mg.ml^{-1} *Carica* acetone and *Syzygium* acetone (5 and 15 mg.ml^{-1}) extracts had the highest emergence percentage in both cowpea cultivars used in the study when compared with other plant extracts. The listed plants extracts that gave the highest emergence percentage were insignificantly (P≤0.05) different from each other and compared well with the non-inoculated control and Celest® XL. The shortest MET was observed in seeds treated with 5 mg.ml^{-1} *Allium* water, 5 and 15 mg.ml^{-1} *Agapanthus* water and 5 mg.ml^{-1} *Carica* acetone extracts. These three mentioned treatments compared well with those of Celest® XL treated and the non-inoculated controls and were significantly (P≤0.05) different from the negative controls and seeds treated with other extracts (Table 4.1).

4.4.2 Bean germination, emergence and mean emergence time

Almost all the bean seeds planted in the 2009 and 2010 seasons treated with the plant extracts had a germination percentage of above 90 % and compared well with Celest® XL treated seeds with exception of 15 mg.ml^{-1}*Agapanthus* acetone (83 %) in 2010 and 15 mg.ml^{-1} *Syzygium* acetone (88.8 and 82.7 %) in 2009 and 2010, respectively (Table 4.2). The seed treatments that gave the highest bean percentage normal seedlings from seeds planted in 2009 and 2010 were 5 mg.ml^{-1} *Allium* water extracts (84.0 and 84.8 %), 5 mg.ml^{-1} *Agapanthus* acetone (86.4 and 86.7 %), *Allium* 5 mg.ml^{-1} (85.1 and 88.0 %) and 15 mg.ml^{-1} (88.8 and 89.3 %) acetone extracts and Celest® XL (87.2 and 93.3 %) treated seeds.

In common bean, the highest emergence percentage for seeds planted in 2009 and 2010 treated with plant extracts was recorded in seeds treated with *Allium* water (5 and 15 mg.ml^{-1}), 5 mg.ml^{-1} *Agapanthus* water, 15 mg.ml^{-1} *Carica* water, *Syzygium* water (5 and 15 mg.ml^{-1}) extracts and *Agapanthus* 5 and 15 mg.ml^{-1} acetone extracts (Table. 4.4). The

emergence percentage of seeds treated with the above mentioned extracts was not significantly different (P≤0.05) from the emergence percentage of Celest® XL and that of the non–inoculated treatment. Surprisingly, the emergence percentage of the inoculated water soaked treatment (91.7 %) was statistically insignificant to that of Celest® XL (83.3 %).

Bean seeds treated with 5 and 15 mg.ml^{-1} water extracts of *Allium*, *Agapanthus*, *Syzygium*, 5 and 15 mg.ml^{-1} of *Agapanthus* and *Carica* acetone and 5 mg.ml^{-1} *Allium* acetone extracts had the shortest mean emergence time (MET) and were not significantly (P≤0.01) different from each other and from both the positive and negative controls (Table 4.2).

4.4.3 Cowpea shoot length, dry mass and leaf area

Cowpea seedling shoot lengths between treatments were significantly different (P≤0.05) at 28 and 42 days after sowing (DAS) in IT93K5132 but not in PAN 311 (Table 4.5). Water extracts of *Agapanthus* (15 mg.ml^{-1}), *Allium* (5 mg.ml^{-1}), *Carica* (15 mg.ml^{-1}), *Syzygium* (5 and 15 mg.ml^{-1}) and acetone extracts of *Carica* and *Syzygium* both at 5 and 15 mg.ml^{-1} concentrations had tallest shoot lengths. The shoot lengths of the treatments listed above were not significantly different (P≤0.05) from those of the positive controls (Celest® XL treated and non-inoculated). The shoot length increased with increased concentrations of *Agapanthus* and *Carica* water plant extracts. *Carica* acetone and *Syzygium* (water and acetone) extracts had the tallest shoots at both low and high concentrations.

The 15 mg.ml^{-1} *Allium* water extracts had the significantly (P≤0.05) highest leaf area for the two cowpea varieties used in the study followed by 15 mg.ml^{-1} (water and acetone) *Syzygium* extracts and the non-inoculated control (Table 4.5). Only the 15 mg.ml^{-1} *Carica* acetone extract had the highest dry mass for both IT93K5132 and Pan 311 cowpea varieties followed by 5 mg.ml^{-1} *Carica* water extracts and had higher dry mass that were significantly (P≤0.05) different from other extracts and the controls (Table 4.5).

4.4.4 Bean shoot length, plant mass (dry mass) and leaf area

The common bean seedling shoot lengths were significantly different (P≤0.05) between seedlings from the 2009 and 2010 seasons at 28 DAS but insignificant in the 2010 bean seeds at 42 DAS (Table 4.6). The seedlings from bean seeds treated with *Agapanthus* 5 mg.ml^{-1} water,15 mg.ml^{-1} *Carica* water and *Agapanthus* acetone 5 and 15 mg.ml^{-1} extracts produced the longest seedlings from both 2009 and 2010 seasons at 28 DAS and compared well with seedlings from Celest® XL treated seeds. The seedlings from seeds treated with all the plant extracts had the tallest plants and compared well with seedlings from the positive control treatments at 42 DAS but were significantly (P≤0.05) taller than the inoculated control and 15 mg.ml^{-1} *Carica* acetone extracts. Both the low and high concentrations of *Agapanthus* acetone, 5 mg.ml^{-1} *Agapanthus* water, *Carica* 15 mg.ml^{-1} water extracts and Celest® XL were consistent in increasing the height of the seedlings from both 2009 and 2010 seeds.

The leaf area for the seedlings from the 2009 and 2010 bean seeds were highly significant (P≤0.01) among treatments. The seedlings from the 2009 and 2010 seeds treated with low concentration (5 mg.ml^{-1}) *Allium* water and *Syzygium* acetone and 15 mg.ml^{-1} *Agapanthus* water extracts had the highest leaf areas (Table 4.6). The above mentioned treatments were insignificant from each other and their leaf areas were significant from those of the two positive controls (Table 4.6).

The seedling dry weight was significant (P≤0.05) among treatments from 2010 bean seeds and insignificant (P≤0.05) for seedlings from 2009 bean seeds. The highest dry mass was observed in seedlings from seeds treated with 5 mg.ml^{-1} *Carica* water, 15 mg.ml^{-1} *Allium* water, 5 mg.ml^{-1} *Syzygium* acetone extracts and DMSO.

4.4.5 Cowpea anthracnose disease incidence and severity

Low cowpea anthracnose disease incidences were observed in seedlings from seeds treated

with 5 and 15 mg.ml^{-1} *Allium*, *Carica*, *Syzygium* water extracts and *Agapanthus*, *Carica* and 15 mg.ml^{-1} *Syzygium* acetone extracts (Fig. 4.1). However, all seeds treated with the plant extracts except 15 mg.ml^{-1} *Allium* acetone extracts resulted in seedlings with low cowpea anthracnose disease severities and compared well significantly (P≤0.05) with the positive controls (non-inoculated treatments and Celest® XL (Fig. 4.2).

4.4.6 Bean anthracnose disease incidence and severity

The *Agapanthus* (5 and 15 mg.ml^{-1}) water, *Allium* (5 and 15 mg.ml^{-1}) acetone, *Carica* acetone (15 mg.ml^{-1}) and *Syzygium* acetone (5 mg.ml^{-1}) extracts treated seeds registered no bean anthracnose disease incidence on seedlings and compared well with the non-inoculated control (Fig. 4.3). The above mentioned seed treatments were however, not significantly different (P≤0.05) from other extracts except those of 15 mg.ml^{-1} *Carica* water extract and the negative controls (DMSO treated and inoculated control). Common bean disease severity was significantly different (P≤0.05). The seedlings from seeds treated with all the plant extracts recorded lower disease severities than the negative controls but *Agapanthus* water extract, Celest® XL and all the seedlings from seeds treated with acetone plant extracts used in this study had the lower disease severity and compared well with the non-inoculated control (Fig. 4.4).

4.5 DISCUSSION

4.5.1 Effect of plant extract seed treatments on cowpea germination, emergence, growth and anthracnose disease incidence and severity

The germination percentage was improved with an increase in concentration of *Allium* acetone and *Syzygium* water extracts as compared with the water control. *Syzygium* water extracts improved the IT93K5232 cowpea seeds germination with an increase in

concentrations and the performance of *S. cordatum* water extracts correlated with the findings of Shafique *et al*. (2007) which indicated that aqueous *S. cumini* extract enhanced wheat (*T. aestivum*) seeds germination. The *Allium* acetone and *Syzygium* water extracts improved the germination of IT93k5132 cowpea seeds when compared to the DMSO and water control. The results were, however, in contrast to the findings of Nwachukwu and Umechuruba (2001) who found that the extracts of *C. papaya* promoted African yam bean seed germination over the water control. The IT93k5132 cowpea seed treated with *Agapanthus* acetone extracts had a higher germination percentage than the water control and this was similar to the findings of WIPO (2007) who reported that the crude extracts of flowers, flower stalks, leaves and aerial parts of *Agapanthus africanus* increased germination of radish (*Raphanus sativus* L.) seeds when compared to the water control. The results revealed that *Allium*, *Carica*, *Syzygium* water extracts and *Allium*, *Agapanthus* acetone extracts promoted germination of cowpea over the negative controls and the mentioned treatments compared well with the positive controls (Celest® XL and non-inoculated control).

High emergence percentage of both IT93k5132 and Pan 311 cowpea seeds were recorded in seeds treated with *Agapanthus* 5 $mg.ml^{-1}$ water extract and the acetone extracts of *Allium* 5 $mg.ml^{-1}$, *Carica* 5 $mg.ml^{-1}$ and *Syzygium* (5 and 15 $mg.ml^{-1}$). There was a slight decline in the emergence percentage at a higher (15 $mg.ml^{-1}$) concentration and this could be attributed to toxicity of the extracts.

The 5 $mg.ml^{-1}$ *Allium* water, *Agapanthus* (5 and 15 $mg.ml^{-1}$) water extracts and positive controls of Pan 311 cowpea treated seeds registered the lower MET. The above treatments are good treatments because they had a higher number of germinated seeds within a short period and escaped the risk of attack by disease-causing organisms or soil pests (Jonitz and Leist, 2003).

Carica water (5 $mg.ml^{-1}$) and *Carica* acetone extracts (15 $mg.ml^{-1}$) gave the highest

dry mass of the two cultivars of cowpea used in this study and these results could be due to lower incidences and severities of anthracnose of cowpea plants from 15 mg.ml^{-1} *Carica* acetone and 5 mg.ml^{-1} *Carica* water extracts treated seeds. Anthracnose disease reduces the photosynthetic leaf area of plants thereby reduces the plant growth rate (Agrios, 2005).

The IT93K5132 and Pan 311 cowpea seedlings from seeds treated with 15 mg.ml^{-1} *Allium* water extract had the highest leaf area due to the antifungal activities of the *Allium* extract against the seed-borne (Islam *et al*., 2001; Obagwu, 2003; Shafique*et al.,* 2007) and soil-borne phytopathogen (Taraq and Magee, 1990; Abdulrahman and Aba Alkhali, 2005) that suppressed all the pathogens that were within and around the seeds. The suppression of phytopathogens resulted in the earliest emergence (short MET) that gave it more time for vegetative growth and the low incidence and severity of anthracnose disease observed in this treatment.

Low cowpea anthracnose disease incidences and severity were observed in seedlings from seeds treated with 5 and 15 mg.ml^{-1} *Allium*, *Carica*, *Syzygium* water extracts and *Agapanthus*, *Carica* and 15 mg.ml^{-1} *Syzygium* acetone extracts. The *in vivo Allium* water extracts results concurred with the *in vitro* findings (Chapter 3) and also with those of Shovan *et al.* (2008) where *A. sativum* water extracts inhibited the growth of *C. dematium*.

4.5.2 Effect of plant extract seed treatments on bean germination, emergence, growth and anthracnose disease incidence and severity

All water extracts, *Allium* and *Carica* acetone (5 and 15 mg.ml^{-1}), and 5 mg.ml^{-1} *Agapanthus* acetone treated bean seeds registered the highest germination percentage that compared well with Celest® XL treated seeds. The plant extracts that had higher germination and normal seedling percentages were reported to possess the antifungal activities that are effective against some seed-borne pathogens like *Alternaria* spp., *Fusarium* spp., *Pythium* spp.,

Rhizoctonia spp. and *Colletotrichum* spp. (Tegegne *et al.*, 2008; Bautista-Ban˜osa, 2003). WIPO (2007) reported that low concentrations of plant extracts promoted seed germination and high concentrations reduced germination and this concurred with the *Agapanthus* acetone treated 2010 bean seed germination results of this experiment. Shafique *et al*. (2007) reported the enhancement of wheat seed germination due to aqueous *Syzygium cumini* extract treatment over the control, which was similar to what was found with the *S. cordatum* water extracts in this study.

The 5 and 15 mg.ml^{-1} water extracts of *Allium*, *Agapanthus*, *Syzygium* and *Carica* and *Agapanthus* acetone extracts and Celest® XL treated common bean seeds recorded the shortest MET. This is indicative of a good seed treatment because seedlings carry a risk of attack by disease-causing organisms or soil pests when MET is short (Jonitz and Leist, 2003).The emergence and MET results suggest that the *Allium* water, *Agapanthus* (acetone and water) and *Syzygium* water extracts have no negative effects on seedlings emergence as well as the mean emergence time.

The tallest seedlings were observed in bean seedlings from seeds both from 2009 and 2010 treated with *Agapanthus* 5 mg.ml^{-1} water, 15 mg.ml^{-1} *Carica* water, *Agapanthus* acetone (5 and 15 mg.ml^{-1}) extracts and Celest® XL at 28 DAS. The bean plants from *Agapanthus* (5 and 15 mg.ml^{-1}) acetone extract and *Agapanthus* (5 mg.ml^{-1}) water extract had the tallest plants due to the lower incidence and severities of anthracnose disease and the low MET that gave the plants more growth period as compared to other treatments that had a longer MET.

Fifteen mg.ml^{-1} *Allium* water,5 mg.ml^{-1} *Carica* water extracts and 5 mg.ml^{-1} *Syzygium* acetone extract and DMSO treated bean seeds produced plants that had the highest dry mass because they had a short MET that gave the seedlings more time for biomass production. The seed treatment that has a short MET has a long growing period of the crop and consequently results in high yield (Jonitz and Leist, 2003). The high biomass by seedlings from *Allium*

water and *Syzygium* acetone extract treated seeds could have also been contributed by the high leaf area recorded.

Bean (Jenny 2009) seedlings from Celest® XL treated seeds had a lower leaf area as compared to other treatments due to common bacterial blight. The incidence of common bacterial blight (*Xanthomonas campestris* pv. *phaseoli* (Smith) Dye – data not included) in seedlings from bean seeds planted in 2009 treated with Celest® XL occurred because Celest® XL is a fungicide and has no bactericidal activity. Common bacterial blight causes leaf spots that enlarge, coalesce and become necrotic thereby reducing the photosynthetic capacity of the plants (Agrios, 2005). The reduced photosynthetic capacity of the leaves leads to the reduced growth of the affected seedlings as less photosynthetic products are translocated to the actively growing parts (Agrios, 2005).

Agapanthus water (5 $mg.ml^{-1}$), *Carica* acetone (15 $mg.ml^{-1}$) and *Syzygium* acetone (5 $mg.ml^{-1}$) treated seeds gave plants that had no bean anthracnose disease incidence and severity. The low incidences and severity of anthracnose were observed in bean plants from seeds treated with acetone extracts of *Agapanthus* (5 and 15 $mg.ml^{-1}$), *Allium* (5 and 15 $mg.ml^{-1}$), *Carica* (5 $mg.ml^{-1}$), *Syzygium* acetone (15 $mg.ml^{-1}$), *Allium* (5 $mg.ml^{-1}$) water extract and Celest® XL and these results were in agreement with the *in vitro* study findings in Chapter 3. The incidences and severity of anthracnose of plants treated with Celest® XL could have been due to the deep seated infections in the seed that sometimes survive the treatment (Dillard, 1988).

Generally, there was a decrease in emergence percentage and increase in MET of both crops (cowpea and bean) as the plant extract concentration was increased from 5 to 15 $mg{\cdot}ml^{-1}$ in most treatments. The decline in the emergence rate could be attributed to toxicity of the extracts. There were some inconsistencies in terms of the effect of extracts on cowpea and bean emergence by other extracts. A particular extract could reduce the emergence of one

variety and promote it in another. Bean seeds obtained in 2010 had higher emergence than bean seeds obtained in 2009 and Pan 311 cowpea seeds recorded higher emergence than IT93K5132. The IT93K5132 cowpea and bean (2009) seeds were both collected in 2009 whereas Pan 311 cowpea and bean (2010) seed were from the 2010 harvest. The low emergence percentage IT93K5132 and bean (2009) could be due to deterioration of their vigour during the storage period (Aveling, 2010).

The *Syzygium* (both water and acetone) extracts showed the lowest cowpea and bean anthracnose disease incidence and severity. The *in vivo* performance of *Syzygium* was in agreement of the microtitre dilution assay (Chapter 3) where it was active against both *C. dematium* and *C. lindemuthianum.* Acetone plant extracts showed the highest inhibitory activity among the other solvents' extracts *in vitro* and also gave good results *in vivo* against *C. lindemuthianum* (bean anthracnose). Similar results pertaining to the higher inhibition of acetone extracts were reported by Masoko *et al.* (2005) who stated that acetone extracts were superior to other extraction solvents such as hexane, dichloromethane and methanol. All the plant extracts except for the *Carica* water extracts were effective in reducing anthracnose disease severity. The anthracnose disease incidences in some seedlings from extracts and Celest® XL treated seeds could be due to the deep seated infections that escaped treatments.

Five $mg.ml^{-1}$ concentrations of *Syzygium* and *Agapanthus* water extracts and acetone extracts of *Agapanthus* and *Carica* have potential as seed treatments on bean. *Allium* acetone extract (5 $mg.ml^{-1}$) is the only potential cowpea seed treatment that could be recommended to farmers as an alternative to the synthetic fungicide. The mentioned treatments had the higher germination percentages, MET, shoot length, leaf area and dry mass of common bean and cowpea. Jonitz and Leist (2003) reported that the fungicidal active ingredients for use in seed treatment are expected to have a rapid action and broad spectrum activity against disease causing organisms and meet extremely high standards with regards to tolerability of the

crops. Further research is required to determine the optimum period needed for seed treatment (soaking) of cowpea seed in order to achieve minimum seed injuries, high germination, high emergence, low MET and low anthracnose disease incidence and severity. There is also a need to further investigate the performance of water and acetone *Allium*, *Agapanthus*, *Carica* and *Syzygium* extracts *in vivo* as foliar fungicides to control anthracnose disease of cowpea.

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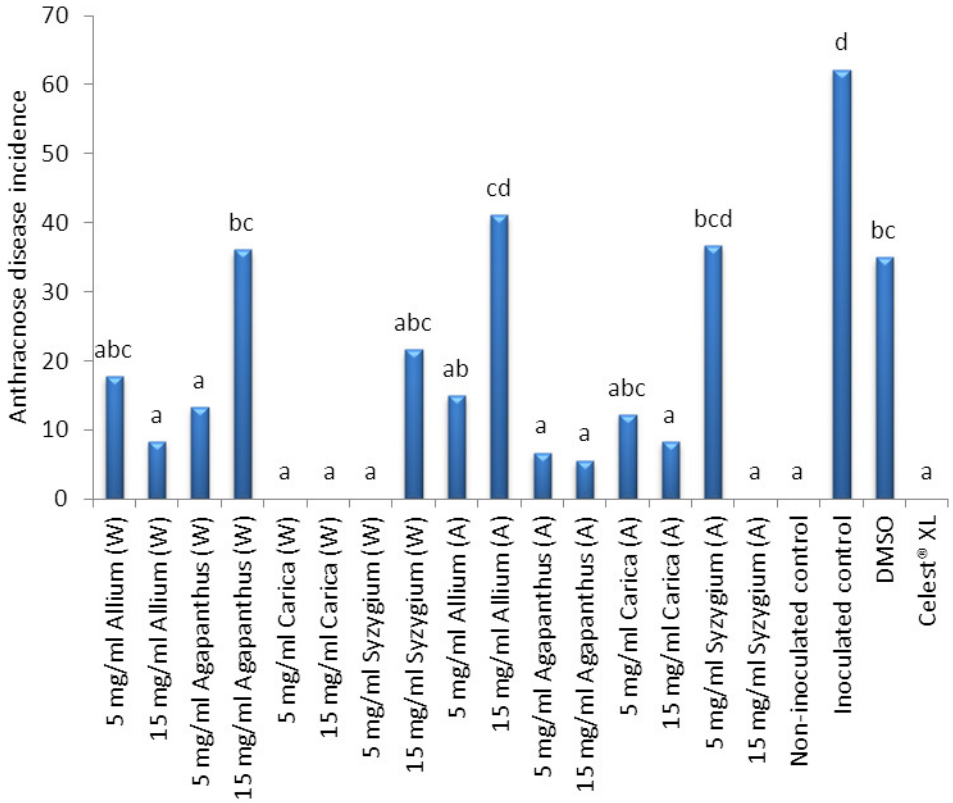

Figure 4.1: Cowpea anthracnose disease incidence from cowpea seeds treated with different plant extracts at 5 and 15 mg.ml^{-1}. A = acetone extracts and W = water extracts. Bars bearing different letters are significantly different at the P≤0.05 level.

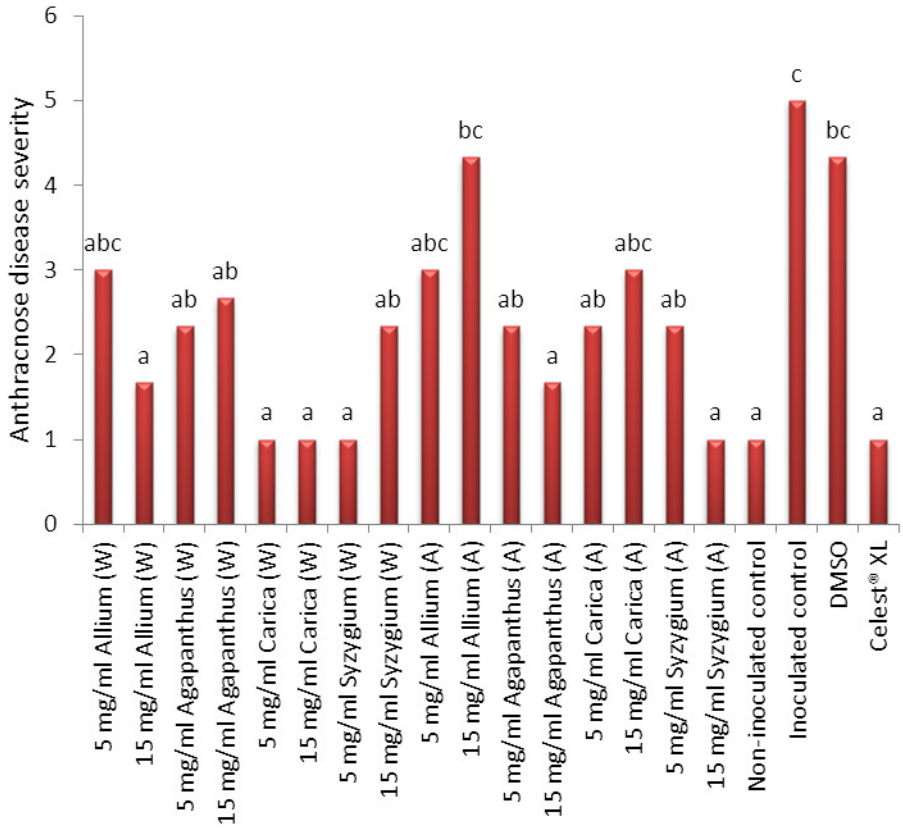

Figure 4.2: Cowpea anthracnose disease severity of cowpea seedlings from cowpea seeds treated with different plant extracts at 5 and 15 mg.ml^{-1}. A = acetone extracts and W = water extracts. Bars bearing different letters are significantly different at the P≤0.05 level.

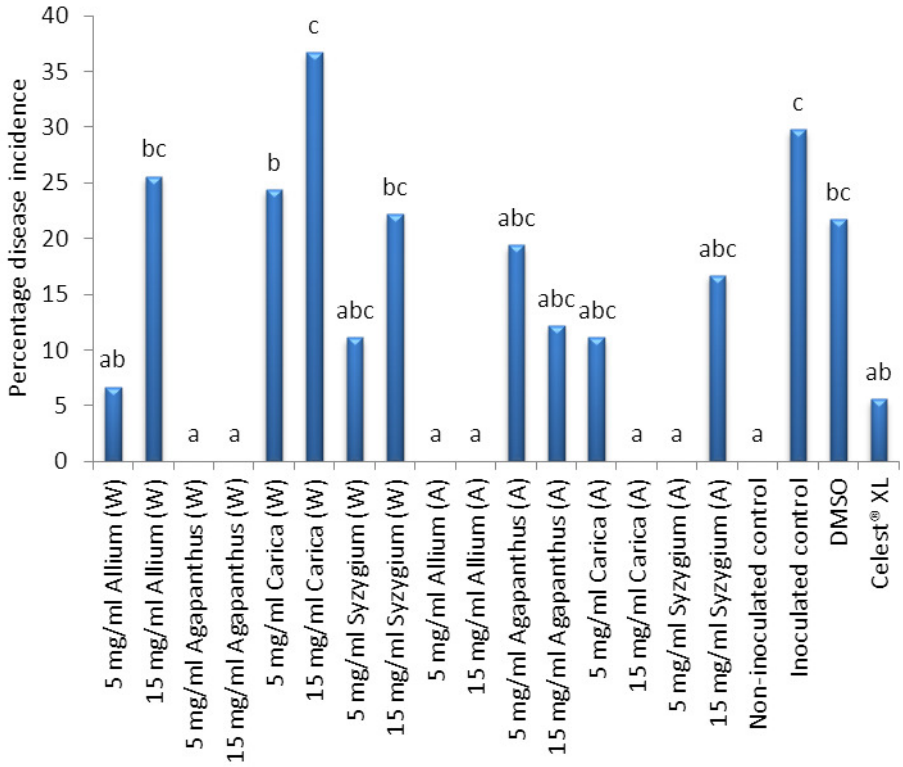

Figure 4.3: Bean anthracnose disease incidence from bean seeds treated with different plant extracts at 5 and 15 mg.ml^{-1}. A = acetone extracts and W = water extracts. Bars bearing different letters are significantly different at the P≤0.05 level.

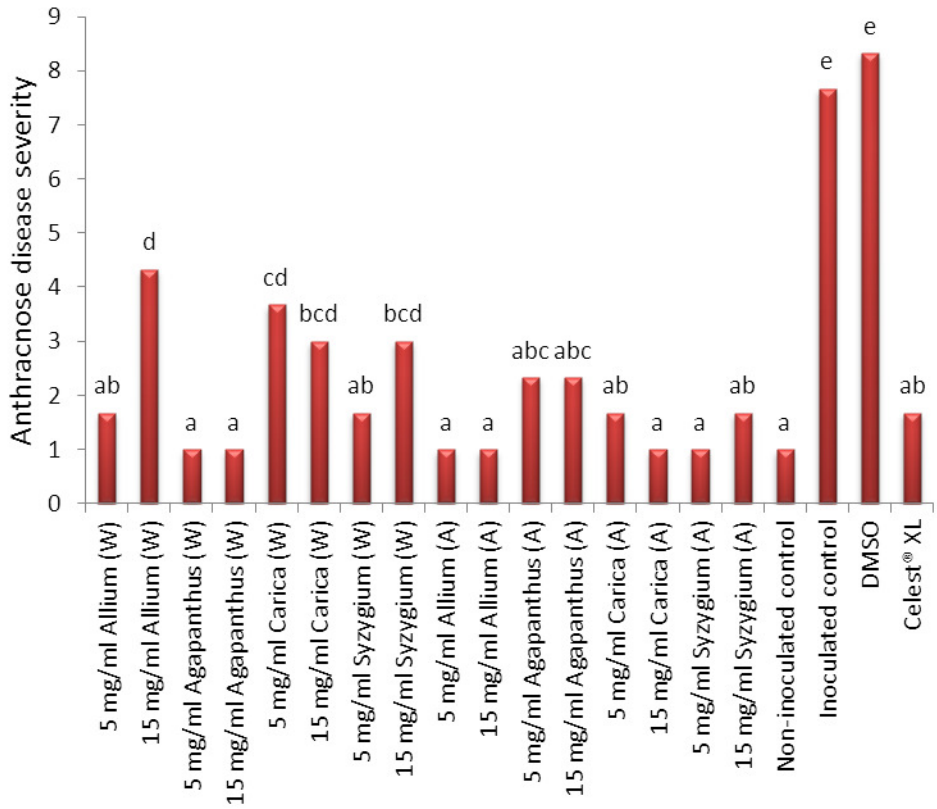

Figure 4.4: Bean anthracnose disease severity of bean seedlings from bean seeds treated with different plant extracts at 5 and 15 mg.ml^{-1}. A = acetone extracts and W = water extracts. Bars bearing different letters are significantly different at the P≤0.05 level.

Table 4.1: Percentage of seed germination, normal seedlings, abnormal seedlings, dead seed and mean emergence time (MET) of IT93K5132 and PAN 311 cowpea seed treated with plant extracts at 5 and 15 mg.ml^{-1}.

	IT93K5132				PAN 311				
Treatment	% germination	% normal seedlings	% abnormal seedlings	% dead seeds	% germination	% normal seedlings	% abnormal seedlings	% dead seeds	Mean emergence time
5 mg/ml *Allium sativum (W)*	91.2 cdef	79.2 d	8.0	8.8	82.7	67.4	15.3	17.3	3.67 ab
15 mg/ml *Allium sativum (W)*	94.4 ef	79.2 d	10.4	5.6	77.3	62.0	15.3	23.3	5.00 bc
5 mg/ml *Agapanthus caulescens (W)*	96.0 f	83.2 d	8.0	4.0	76.0	66.0	10.0	24.0	3.67 ab
15 mg/ml *Agapanthus caulescens (W)*	84.8 abcd	76.8 bcd	6.4	15.2	87.3	74.7	12.7	12.7	4.67 abc
5 mg/ml *Carica papaya (W)*	91.2 cdef	80.0 d	1.6	7.2	72.7	66.7	6.0	27.3	5.33 bcd
15 mg/ml *Carica papaya (W)*	92.8 def	82.4 d	4.8	7.2	82.7	73.4	9.3	17.3	6.00 cdef
5 mg/ml *Syzygium cordatum (W)*	85.6 abcd	78.4 bcd	4.0	14.4	75.3	68.0	7.3	24.7	7.33 ef
15 mg/ml *Syzygium cordatum (W)*	88.0 bcdef	76.8 bcd	5.6	12.0	77.3	67.3	10.0	22.7	7.33 ef
5 mg/ml *Allium sativum (A)*	87.2 bcde	76.8 bcd	6.4	12.8	72.7	60.7	12.0	27.3	7.00 def
15 mg/ml *Allium sativum (A)*	88.8 bcdef	80.0 d	5.6	11.2	82.7	66.0	16.7	17.3	7.00 def
5 mg/ml Agapanthus caulescens (A)	91.2 cdef	82.4 d	6.4	8.0	84.7	73.4	11.3	15.3	6.00 cdef
15 mg/ml *Agapanthus caulescens (A)*	90.4 bcdef	80.8 d	5.6	9.6	80.7	68.0	12.7	19.3	5.33 bcd
5 mg/ml *Carica papaya (A)*	84.0 abc	73.6 bcd	8.8	16.0	71.3	52.0	19.3	28.7	4.67 abc
15 mg/ml *Carica papaya (A)*	86.4 abcde	76.0 bcd	7.2	13.6	71.3	60.0	11.3	28.7	7.67 f
5 mg/ml *Syzygium cordatum (A)*	88.0 bcdef	73.6 bcd	8.0	12.0	77.3	62.0	15.3	22.7	5.00 bc
15 mg/ml *Syzygium cordatum (A)*	78.4 a	68.0 abc	14.4	20.8	79.3	64.0	15.3	20.7	5.33 bcd
Water	82.4 ab	66.4 ab	8.0	17.6	74.7	61.4	13.3	25.3	3.00 a
1% DMSO	83.2 abc	60.8a	16.8	16.8	70.7	56.7	14.0	29.3	5.00 bc
Celest® XL	90.4 bcdef	77.6 cd	12.8	9.6	75.3	66.7	8.7	24.7	4.67 abc
Inoculated control									5.67 cde
Significant level	*	**	NS	*	NS	NS	NS	NS	**
LSD	8.66	10.92	1.50	1.38	12.12	12.05	1.90	3.47	1.778

A = acetone extracts; W = water extracts; NS = not significant different at P≤0.05
* = significant at P≤0.05; ** = significant at P≤0.01. The figures within the same column bearing same letters are not significantly different at the P≤0.05 or P≥0.01.

Table 4.2: Percentage of seed germination, normal seedlings, abnormal seedlings and dead seed of common bean treated with plant extracts at 5 and 15 mg.ml^{-1}.

Treatment	2009 Bean Crop				2010 Bean Crop				Mean emergence time
	% germination	% normal seedlings	% abnormal seedlings	% dead seeds	% germination	% normal seedlings	% abnormal seedlings	% dead seeds	
5 mg/ml *Allium sativum (W)*	94.8 bc	84.8 bcde	10.0	5.2 abc	99.3 c	84.0 defghi	9.6 abc	6.7 abc	6.50 abc
15 mg/ml *Allium sativum (W)*	95.13 bc	87.06 bcde	8.1	5.67 abc	92.7 bc	79.3 defg	18.7 efg	7.3 abc	7.50 abc
5 mg/ml *Agapanthus caulescens (W)*	97.6 c	89.6 de	8.0	3.2 ab	99.3 c	79.7 defg	20.2 fgh	0.7 ab	5.75 a
15 mg/ml *Agapanthus caulescens (W)*	97.6 c	89.4 de	8.2	2.4 a	100.0 c	78.3 cdef	21.3 gh	0.0 a	6.25 ab
5 mg/ml *Carica papaya (W)*	97.6 c	89.6 de	8.0	4.0 ab	100.0 c	81.3 defg	18.7 efg	0.0 a	6.25 ab
15 mg/ml *Carica papaya (W)*	92.0 bc	80.0 abc	10.4	8.0 abcd	95.3 c	75.3 bcd	20.0 fgh	4.7 ab	8.50 c
5 mg/ml *Syzygium cordatum (W)*	96.0 bc	92.0 e	4.0	4.0 ab	100.0 c	78.7 cdef	14.7 bcdef	0.0 a	7.00 abc
15 mg/ml *Syzygium cordatum (W)*	96.8 cb	88.0 bcde	8.8	3.2 ab	91.8 c	77.5 cde	14.3 bcdef	8.2 bc	6.25 ab
5 mg/ml *Allium sativum (A)*	90.87 b	85.1 bcde	5.8	9.1 bcd	99.3 c	88.0 fghi	11.3 abcd	0.7 ab	5.75 a
15 mg/ml *Allium sativum (A)*	93.6 bc	88.8 cde	4.8	6.4 abc	98.0 c	89.3 ghi	8.7 abc	2.0 ab	8.00 bc
5 mg/ml Agapanthus caulescens (A)	91.2 bc	86.4 bcde	4.8	8.8 bcd	99.3 c	86.7 efghi	12.7 abcde	0.7 ab	5.75 a
15 mg/ml *Agapanthus caulescens (A)*	96.0 bc	90.4 de	5.6	14.0 de	83.0 a	69.3 a	12.7 abcde	18.0 d	6.50 abc
5 mg/ml *Carica papaya (A)*	92.0 bc	88.0 bcde	4.0	8.0 abcd	99.3 c	86.0 efgh	13.3 bcd	0.7 ab	7.00 abc
15 mg/ml *Carica papaya (A)*	94.4 bc	89.6 de	4.8	5.6 abc	95.3 c	67.3 ab	28.0 i	4.7 ab	5.50 a
5 mg/ml *Syzygium cordatum (A)*	96.0 bc	88.8 bcde	7.2	4.0 abc	100 c	82.7 defgh	17.3 defg	0.0 a	6.25 ab
15 mg/ml *Syzygium cordatum (A)*	88.8 b	75.2 ab	13.6	11.2 cd	82.7 a	60.7 a	25.3 hi	16.7 d	12.25 d
Water	93.6 bc	82.4 bcde	11.2	6.4 abc	87.0 ab	69.0 abc	18.04 ef	12.97 d	5.75 a
1% DMSO	79.2 a	71.2 a	8.0	20.8 e	100.0 c	91.6 hi	8.44 abc	0.0 a	6.25 ab
Celest® XL	93.6 bc	87.2 bcde	5.6	6.4 abc	100.0 c	93.3 i	6.7 a	0.0 a	6.00 ab
Inoculated control									6.25 ab
Significant level	**	**	NS	**	**	**	**	**	**
LSD	6.43	8.92	1.10	2.48	7.998	10.136	2.54	2.84	2.10

A = acetone extracts; W = water extracts; * = significant at P≤0.05; ** = significant at P≤0.01. The figures within the same column bearing same letters are not significantly different at the P≤0.05 or P≥0.01 level.

Table 4.3: The emergence percentage of cowpea treated with different plant extracts at 5 and 15 mg.ml^{-1}.

	Cowpea	
Treatments	IT93K5132	PAN311
5mg/ml *Allium sativum (W)*	44.4 abc	83.3 cde
15mg/ml *Allium sativum (W)*	55.6 bcd	72.2 bcd
5mg/ml *Agapanthus caulescens (W)*	55.6 bcd	77.8 bcde
15mg/ml *Agapanthus caulescens (W)*	77.8 cd	61.1 ab
5mg/ml *Carica papaya (W)*	88.9 d	66.7 bc
15mg/ml *Carica papaya (W)*	77.8 cd	72.2 bcd
5mg/ml *Syzygium cordatum (W)*	66.7 bcd	72.2 bcd
15mg/ml *Syzygium cordatum (W)*	44.4 abc	72.2 bcd
5mg/ml *Allium sativum (A)*	77.8 cd	77.8 bcde
15mg/ml *Allium sativum (A)*	44.4 abc	66.7 bc
5mg/ml *Agapanthus caulescens (A)*	33.9 ab	83.3 cde
15mg/ml *Agapanthus caulescens (A)*	11.1 a	77.8 bcde
5mg/ml *Carica papaya (A)*	66.7 bcd	83.3 cde
15mg/ml *Carica papaya (A)*	55.5 bcd	66.7 bc
5mg/ml *Syzygium cordatum* (A)	77.8 cd	94.4 e
15mg/ml *Syzygium cordatum* (A)	88.9 d	77.8 bcde
Water (Non inoculated)	88.9 d	94.4 e
Inoculated water control	44.4 abc	44.4 a
DMSO	44.4 abc	77.8 bcde
Celest® XL	88.9 d	83.3 cde
Significance	*	**
LSD	39.22	17.76

A = acetone extracts; W = water extracts; * = significant at P≤0.05;
** = significant at P≤0.01. The figures within the same column bearing same letters are not significantly different at the P≤0.05 or P≥0.01.

Table 4.4: The emergence percentage of common bean treated with different plant extracts at 5 and 15 mg.ml^{-1}.

	Bean	
Treatments	2009 crop	2010 crop
5 mg/ml *Allium sativum* (W)	75.0 bcd	100.0 c
15 mg/ml *Allium sativum* (W)	91.7 d	92.2 c
5 mg/ml *Agapanthus caulescens* (W)	91.7 d	100.0 c
15 mg/ml *Agapanthus caulescens* (W)	66.7 bcd	73.9 a
5 mg/ml *Carica papaya* (W)	58.3 abc	96.1 c
15 mg/ml *Carica papaya* (W)	75.0 bcd	96.1 c
5 mg/ml *Syzygium cordatum* (W)	83.3 cd	92.2 c
15 mg/ml *Syzygium cordatum* (W)	75.0 bcd	92.2 c
5 mg/ml *Allium sativum* (A)	nt	92.2 c
15 mg/ml *Allium sativum* (A)	58.3 abc	100.0 c
5 mg/ml *Agapanthus caulescens* (A)	83.3 cd	88.9 bc
15 mg/ml *Agapanthus caulescens* (A)	75.0 bcd	96.1 c
5 mg/ml *Carica papaya* (A)	nt	100.0 c
15 mg/ml *Carica papaya* (A)	50.0 ab	100.0 c
5 mg/ml *Syzygium cordatum* (A)	33.3 a	96.1 c
15 mg/ml *Syzygium cordatum* (A)	75.0 bcd	75.5 ab
Water (Non inoculated)	83.3 cd	100.0 c
Inoculated water control	75.0 bcd	88.3 bc
DMSO	83.3 cd	96.1 c
Celest® XL	91.7 d	100.0 c
Significance	*	*
LSD	28.36	14.14

A = acetone extracts; W = water extracts; * = significant at P≤0.05.
The figures within the same column bearing same letters are not significantly different at the P≤0.05.

Table 4.5: The shoot length, leaf area and dry weight of cowpea seedlings from plant extract treated seed.

Treatments	Shoot length (mm)				Leaf area ($cm^{2)}$		Dry weight (g)	
	IT93K5132		PAN 311					
	28 DAS	42 DAS	28 DAS	42 DAS	IT93K5132	PAN311	IT93K5132	PAN311
5 mg/ml *Allium sativum (W)*	83.2 abcde	100.5 abcd	166.2	185.5	19.4 abcde	22.29 a	0.22 abcd	0.52 a
15 mg/ml *Allium sativum (W)*	62.3 ab	82.0 ab	147.2	206.8	24.7 bcde	58.92 e	0.11 ab	0.94 cd
5 mg/ml *Agapanthus caulescens (W)*	63.7 ab	72.7 a	147.5	166.2	11.5 abc	28.25 abc	0.16 abc	0.58 a
15 mg/ml *Agapanthus caulescens (W)*	89.5 abcde	106.5 bcd	155.5	164.2	26.2 bcde	25.78 ab	0.17 abcd	0.59 a
5 mg/ml *Carica papaya (W)*	75.2 abcd	98.1 abcd	145.5	163.8	18.8 abcde	29.47 abc	0.18 abcd	1.05 d
15 mg/ml *Carica papaya (W)*	105.9 de	118.4 cd	180.8	197.8	31.0 cde	27.73 abc	0.21 bcde	0.60 a
5 mg/ml *Syzygium cordatum (W)*	97.8 cde	114.5 cd	163.8	175.0	24.7 bcde	27.05 ab	0.26 bcde	0.68 abc
15 mg/ml *Syzygium cordatum (W)*	96.7 cde	110.3 bcd	165.0	185.5	21.1 bcde	31.07 abcd	0.27 bcde	0.62 ab
5 mg/ml *Allium sativum (A)*	57.8 a	71.5 a	172.5	168.0	17.3 abcd	41.48 d	0.12 abc	0.89 bcd
15 mg/ml *Allium sativum (A)*	68.7 ab	85.7 abc	141.2	145.5	15.6 abc	30.53 abcd	0.17 abcd	0.68 abc
5 mg/ml Agapanthus caulescens (A)	60.0 a	81.3 ab	156.8	192.5	8.9 ab	25.03 ab	0.07 a	0.57 a
15 mg/ml *Agapanthus caulescens (A)*	d	d	170.2	180.5	1.3 a	39.46 cd	0.13 ab	0.77 abc
5 mg/ml *Carica papaya (A)*	81.7 abcde	112.6 bcd	146.5	152.5	31.2 de	25.57 ab	0.29 bcde	0.60 a
15 mg/ml *Carica papaya (A)*	101.4 cde	117.8 cd	154.8	173.8	31.6 de	28.02 abc	0.31 cde	0.78 abcd
5 mg/ml *Syzygium cordatum (A)*	95.0 bcde	106.1 bcd	161.2	172.2	24.7 bcde	25.59 ab	0.22 abcd	0.62 ab
15 mg/ml *Syzygium cordatum (A)*	93.8 bcde	114.4 cd	143.2	157.2	29.4 cde	30.36 abcd	0.30 cde	0.58 a
Water	90.5 abcde	116.6 cd	158.2	207.0	35.1 de	35.19 bcd	0.45 e	0.67 abc
1% DMSO	71.5 abc	105.5 bcd	163.8	176.2	23.7 bcde	33.69 abcd	0.24bcde	0.69 abc
Celest® XL	86.7 abcde	108.7 bcd	148.2	182.0	22.4 bcde	26.89 ab	0.22 abcd	0.51 a
Inoculated control	110.4 e	128.7 d	149.5	161.8	38.2 e	29.34 abc	0.36 de	0.65 ab
Significant level	*	*	ns	ns	*	**	*	*
LSD	32.86	32.06	34.64	53.44	19.49	11.89	0.187	0.27

A = acetone extracts; d = dead; w = water extracts; ns = not significant at P≤0.05;
* = significant at P≤0.05; ** = significant at P≤0.01. The figures within the same column bearing same letters are not significantly different at the P≤0.05 or P≥0.01.

Table 4.6: The shoot length, leaf area and dry weight of common bean seedlings from plant extract treated seed.

Treatments	Shoot length (mm)				Leaf area (cm^2)		Dry weight (g)	
	2009 crop		2010 crop					
	28 DAS	42 DAS	28 DAS	42 DAS	2009 Crop	2010 Crop	2009 Crop	2010 Crop
5 mg/ml *Allium sativum (W)*	123.6 cd	156.1 b	152.2 cde	218	51.9 cde	174.1 d	0.66	1.52 e
15 mg/ml *Allium sativum (W)*	112.0 bcd	157.3 b	142.6 abcd	220.7	38.3 bc	167.5 cd	0.57	1.81 f
5 mg/ml *Agapanthus caulescens (W)*	116.9 bcd	138.0 b	157.2 bcdef	210.2	42.9 cd	101.2 ab	0.61	1.23 bcde
15 mg/ml *Agapanthus caulescens (W)*	100.6 bc	140.6 b	165.3 def	223.1	49.1 cde	128.2 bcd	0.58	1.22 bcde
5 mg/ml *Carica papaya (W)*	129.2 d	148.3 b	148.0 abcde	197.5	43.8 cd	114.1 ab	0.62	1.35 cdef
15 mg/ml *Carica papaya (W)*	107.5 bcd	132.7 b	158.4 bcdef	200.5	34.5 bc	72.1 a	0.46	0.89 abc
5 mg/ml *Syzygium cordatum (W)*	128.9 d	160.2 b	132.5 ab	191.0	59.2 e	86.4 ab	0.63	1.03 abc
15 mg/ml *Syzygium cordatum (W)*	116.1 bcd	141.1 b	139.0 abc	199.9	40.8 cd	87.3 ab	0.52	0.97 abc
5 mg/ml *Allium sativum (A)*	nt	nt	146.4 abcd	200.1	nt	134.0 bcd	nt	1.23 bcde
15 mg/ml *Allium sativum (A)*	107.6 bcd	146.3 b	149.8 abcde	205.5	47.9 cde	85.5 ab	0.58	1.04 abcd
5 mg/ml Agapanthus caulescens (A)	122.9 bcd	161.4 b	172.7 ef	212.6	54.4 de	100.2 ab	0.64	1.21 bcde
15 mg/ml *Agapanthus caulescens (A)*	130.3 d	163.4 b	160.7 cdef	201.3	43.8 cd	101.3 ab	0.58	1.14 abcde
5 mg/ml *Carica papaya (A)*	nt	nt	137.4 abc	196.9	nt	103.2 ab	nt	1.22 bcde
15 mg/ml *Carica papaya (A)*	71.1 a	99.3 a	143.1 abcd	193.1	19.5 a	61.9 a	0.26	0.71 a
5 mg/ml *Syzygium cordatum (A)*	96.7 ab	159.2 b	162.5 cde	213.1	46.7 cde	164.4 cd	0.59	1.77
15 mg/ml *Syzygium cordatum (A)*	112.9 bcd	142.7 b	129.4 a	165.7	45.3 cde	71.0 a	0.52	0.82 ab
Water	99.0 bc	137.0 b	182.9 f	224.4	38.2 bc	139.3 bcd	0.47	1.21 bcde
1% DMSO	107.5 bcd	127.3 ab	144.9 abcd	214.6	38.3 bc	101.0 ab	nt	1.01 abcd
Celest® XL	114.3 bcd	139.6 b	158.2 bcdef	206.6	38.0 bc	105.5 ab	0.62	1.45 def
Inoculated control	127.7 d	137.1 b	167.3 def	210.9	26.2 ab	117.1 abc	0.51	1.19 bcde
Significant level	*	*	*	ns	**	**	ns	**
LSD	26.77	30.91	26.00	33.46	13.92	55.50	0.77	0.464

A = acetone extracts; w = water extracts; ns = not significant at P≤0.05; nt = not tested;
* = significant at P≤0.05; ** = significant at P≤0.01. The figures within the same column bearing same letters are not significantly different at the P≤0.05 or P≥0.01

CHAPTER FIVE

THE ULTRA-STRUCTURAL CHANGES OF *PHASEOLUS VULGARIS* L. AND *VIGNA UNGUICULATA* L. WALP SEEDS TREATED WITH PLANT EXTRACTS

5.1 ABSTRACT

The changes in the ultra-structure of embryonic roots and the connecting tissues of embryo-cotyledon of common bean (*Phaseolus vulgaris* L.) and cowpea (*Vigna unguiculata* L. Walp) seed treated with *Syzygium cordatum* Hochst.ex Krauss acetone extracts and *Agapanthus caulescens* Spreng water extracts were investigated. The seeds were treated by soaking in 15 $mg.ml^{-1}$ of the plant extracts and the control seeds were soaked in water for 24 h in the dark at 24±2^{o}C. Light and transmission electron microscopy were used to study the differences between the treated and untreated tissue. The principle differences were observed in the cotyledon-embryo connecting tissues of seeds treated with *Agapanthus* which had fewer cristae in their mitochondria than the cells from other treatments. Furthermore, the embryonic root cells of bean seeds treated with *Agapanthus* showed coalescing protein bodies which were not present in the untreated cells. The cells of cowpea and bean seeds treated with *Syzygium* had fewer lipid bodies compared with the control and the *Agapanthus* extract treated seeds.

Key words: bean and cowpea seeds, plant extracts, ultra-structure, embryonic root, cotyledon-embryo connecting tissue

5.2 INTRODUCTION

Seed treatment is reported to be an important process that provides insurance to seed against seed-borne as well as the soil-borne plant pathogens and insects (Bierman *et al.*, 2006; Gwary *et al*., 2007). Seed treatment is relatively environmentally friendly as it reduces contamination of the environment by large volumes of foliar pesticides and fungicides applied whenever disease occurs in the field (Anonymous, 1992). Some seed treatment techniques enhance seed germination, seed lot emergence uniformity and the ability of the seedlings to thrive under unfavourable field conditions (Coolbear and McGill, 1990; Amooaghaie, 2011).

Most of the crops whose seeds are treated are cotton (*Gossypium hirsutum* L.), maize (*Zea mays* L.) and some vegetable seed. Apron® XL (mefenoxam), Apron® Star 42 WS (difenoconazale, metalaxyl-m, and thiamethoxam), Celest® XL (fludioxonil and mefenoxam) and Thiram (tetramethylthiuram disulfide) are some of the chemicals that are commonly used by the seed industry to treat seeds. Some of the compounds found in some of the chemicals listed above such as fludioxonil have been reported to accelerate germination (Cahill, 2000) and thiram has been found to promote viability and emergence of maize seed (Pinto, 1997; Govender, 2008). Csinos (2004) reported that mefenoxam gave the highest vigour of tobacco (*Nicotiana tabacum* L.) when evaluated against tobacco blackshank. Seed treatment with Apron Plus, Almithio and Aldrex T is reported to increase soybean (*Glycine max* L. Merril) seed storage life (Adebisi *et al*., 2004).

Cowpea (*Vigna unguiculata* L. Walp.) and common bean (*Phaseolus vulgaris* L.) have many seed-borne as well as soil-borne diseases but they are rarely treated before sowing. The synthetic chemical fungicides that were reported to be effective against bean anthracnose disease as seed treatments are captan-thiram-metalaxyl-benomyl (CTMB),

diazinon-captan-thiophanate methyl (DCT), thiram-metalaxyl-thiabendazole (TMZ) and carbathiin-thiram (Anchor) (Tu and Zheng, 1994). Inspite of being effective in controlling plant diseases many disadvantages related to the use of synthetic fungicides have been reported by several researchers (Crissman *et al.*, 1996; Anonymous, 2009). Tangley (1987) and Crissman and his co workers (1996) reported the toxicity of agricultural fungicides to humans. The study by Avenot and Michailides (2007) revealed the resistance of a phytopathogen, *Alternaria alternata* to Boscalid, a carboxamide fungicide. The resistance of human fungi to fungicides due to the consumption of agricultural products infected with agricultural fungi that developed resistance to agricultural fungicides has also been reported (Anonymous 2009). The greatest environmental impact of fungicides is toxicity to soil microorganisms as well as the environment (Edwards, 2010). There is also a high demand for organic agricultural products and there is a need to search for environmentally and human friendly fungicides to be used as seed treatments. It is against this background that more scientists are searching for alternative fungicides to be used to control plant diseases.

Many workers have tested the efficacy of different plant extracts as an alternative seed treatment to the synthetic fungicides against soil- and seed-borne pathogens on cereal crops (Shafique *et al.,* 2007; Somda *et al.*, 2007; Sengupta *et al*., 2008), vegetable crops (Alabi *et al*., 2005; Raghavendra *et al.,*. 2006; Akinbode and Ikotun, 2008) and cash industrial crops (Islam *et al.*, 2001) by soaking them in extracts for various periods of time.

Some studies on the ultra-structural changes in seed treated with synthetic fungicides have been reported (Rascio *et al.*, 1993; Govender, 2008). Most of the synthetic fungicide seed treatment is by slurry while most plant extract seed treatment is mainly by soaking despite reports of imbibition damage to some seeds when soaked in water. To date there has been no research on the ultra-structural changes within common bean and cowpea seed treated with plant extracts by soaking and hence this was the aim of this study.

5.3 MATERIALS AND METHODS

5.3.1 Preparation of plant extracts and seed treatment

A concentration of 15 mg.ml^{-1} of *Syzygium cordatum* Hochst.ex Krauss acetone extracts and *Agapanthus caulescens* Spreng water extracts were prepared as described in Chapter 3. Common bean (cv. Jenny) and cowpea (cv Pan 311) seeds were soaked in the water and acetone plant extracts that were diluted to the concentration of 15 mg.ml^{-1} for 24 h in the dark at 24±2 ^{o}C. Seeds soaked in distilled water served as the control.

5.3.2 Light microscopy (LM)

To study the cellular changes in common bean and cowpea post seed treatment by soaking them in two different plant extracts at a concentration of 15 mg.ml^{-1}, the embryonic root (radicle) and the embryo-cotyledon connecting tissues measuring 1mm were prepared from both treated and untreated seeds. The samples were fixed in phosphate-buffered glutaraldehyde (2.5%) for 2 h at room temperature. The fixed samples were rinsed three times for 10 min each in 0.075M phosphate buffer followed by dehydration in ethanol (30, 50, 70, 90, 100, 100, 100 %) for 10 min in each concentration. The infiltration was done in 60% Quetol in ethanol for 1 h and then for 4 h in pure Quetol. The infiltrated materials were embedded in 100% epoxy resin and polymerized in an oven at 60 ^{o}C for 60 h.

Thick sections of 80 nm were cut with a glass knife on a Reichert Ultracut E ultramicrotome (Vienna, Austria), followed by staining with toluidine blue. Observations for differences on the cells were done under a Nikon Optiphot transmitted light microscope (Tokyo, Japan).

5.3.3 Transmission electron microscopy (TEM)

To study the ultra-structural changes in embryonic tissue cells of common bean and cowpea

seeds caused by plant extract treatments, the preparation of 1 mm sections of radicle and embryonic-connecting tissues were done as those for LM. The radicle and embryonic-connecting tissues of the control seeds were also prepared like seeds treated with plant extracts. The ultra-thin sections of radicle tissues and the connecting tissues of cotyledons and embryos were prepared for TEM using a diamond knife on a Reichert Ultracut E ultramicrotome (Vienna, Austria). Ultra-thin sections were then placed on copper grids and contrasted with aqueous uranyl acetate and Reynold's lead citrate for 4 and 2 min, respectively, followed by rinsing in distilled water. Examination of sections was done using a JEM 2100F (JEOL - Tokyo, Japan) transmission electron microscope at 200 KeV.

5.4 RESULTS AND DISCUSSION

There were no differences between cells of the embryonic roots or connecting tissue cells of common bean and cowpea seeds treated with different extracts or distilled water when observed using light microscopy. However, notable differences between treatments were visible when viewing with the TEM.

5.4.1 Embryonic roots of common bean seed treated with *Syzygium* acetone and *Agapanthus* water extracts

The cells of embryonic roots of bean seeds soaked in water (control) had oval mitochondria with many cristae and the endoplasmic reticulum (ER) were linearly arranged (Fig. 5.1A). The cells of the radicle of common bean seed treated with *Syzygium* extracts had few lipid bodies (Fig. 5.1C) when compared to the control (Fig. 5.1A) and those soaked in the *Agapanthus* extracts (Fig. 5.1B). The results revealed that germination had already started in *Syzygium* treated seeds as reported by Hodson and co-workers (1987) and Gunning and Steer (1996) whose studies showed that the layer of lipid bodies gradually disappeared from near

the plasma membrane and were incorporated into the plasma membrane during germination process. The germination rate of *Syzygium* treated seeds was high as compared to the control in Chapter 4 and *Agapanthus* treated seeds as revealed by the faster rate of incorporation of lipid bodies into the plasma membrane. Mollenhauer and Totten (1971) reported that the rate of lipid transformation is proportional to seed germination rate.

The common bean embryonic roots from *Agapanthus* (Fig. 5.1B) and *Syzygium* (Fig. 5.1C) extracts treated seeds had vacuoles but the vacuoles were absent in the common bean embryonic root cells of the water soaked common bean (Fig. 5.1.A). According to De Castro and Martinez-Hounduvilla (1984) vacuoles are present in metabolically active cells as they replace protein bodies during imbibition. In the study by Vijayaraghavan and Jain (1984) it was reported that proteolysis indicates that some controlling factors are operating within the seeds during germination. The collapsing protein bodies (CPB) were observed to coalesce to form one large irregular shaped body in the embryonic root cells of bean seed treated with *Agapanthus* and this coalescence could be the sign of damage that could be suspected to be due to extract injury. The *Agapanthus* extract might have disrupted the protein bodies' integrity there by making them to join. Salts have also been implicated in altering the integrity of cell membrane by inserting new particles in the cell membrane and they do pass through the membrane into the cytoplasm where they are suspected to have toxic effects on cell (Bliss *et al.* 1984).This study indicated that *Agapanthus* extract common bean seed treatment at a higher concentration 15 $mg.ml^{-1}$ can negatively affect the seed germination process by disrupting the protein bodies. The disruption of the protein bodies could be a reason for the low percentage germination, emergence and higher emergence time (MET) of bean seed compared with the low (5 $mg.ml^{-1}$) concentration of the *Agapanthus* extract treated bean seeds observed in Chapter 4.

5.4.2 Cotyledon-embryo connecting tissue of common bean seed treated with *Syzygium* acetone and *Agapanthus* water extracts

The cells of the cotyledon-embryo connecting tissues of bean seeds soaked in distilled water had many lipid bodies that were evenly distributed within the cytoplasm of the cell (Fig. 5.2A) whereas in the *Agapanthus* treated bean seed (Fig. 5.2B) the lipids were aligned along the cell wall and were few in number. The connecting tissue cells of *Syzygium* treated bean seed had no lipid bodies in the cytoplasm or along the cell walls (Fig. 5.2C). Hodson *et al.* (1987) found that the lipid bodies near the plasma membrane of maize (*Zea mays* L.) seeds gradually disappear and become incorporated into the plasma membrane. The absence of the lipid bodies in the cotyledon-embryo connecting tissue cells of *Syzygium* treated bean means that proteolysis and lipolysis was faster than in the *Agapanthus* treated and water soaked bean seed. Proteolysis and lipolysis occurs during imbibition but the former takes place more rapidly than the latter (De Castro and Martinez-Hounduvilla, 1984). The *Agapanthus* extract could have been responsible for the aligning of the lipid bodies along the cell walls of the cotyledon-embryo connecting tissue of *Agapanthus* treated seeds. The study by Bliss *et al.* (1984) reported that salts have also been responsible for altering the integrity of the cell membrane by inserting new particles in the membrane and the salts pass through the membrane into the cytoplasm where they are suspected to have toxic effects on cells. The cotyledon-embryo connecting tissue cells of *Syzygium* treated seeds had mitochondria with many well-formed cristae (Fig. 5.2C) and no cristae were observed in *Agapanthus* treated (Fig. 5.2B) and water soaked seeds (Fig. 5.2A). The rapid development of numerous cristae in the mitochondria of cotyledon-embryo connecting tissue cells of seeds treated with *Syzygium* may be related to rapid initiation of respiration in seeds (Webster and Leopald, 1977).

5.4.3 Embryonic roots of cowpea seed treated with *Syzygium* acetone and *Agapanthus* water extracts

The embryonic roots of cowpea seeds soaked in water (control) had many lipid bodies and protein bodies uniformly distributed within the cell cytoplasm (Fig. 5.3A) and not restricted to the cell wall. The uniformly distribution of protein bodies throughout the cytoplasm were also observed in the embryo cells of wheat (*Triticum aestivum* L.) (Anderson *et al*., 1970). The embryonic roots of cowpea seeds treated with *Syzygium* extract (Fig. 5.3C) and *Agapanthus* extract (Fig. 5.3B) had well developed mitochondria with visible cristae and few lipid bodies than those soaked in water. The presence of cristae and few lipids bodies in the mitochondria of the embryonic roots of cowpea seeds treated with *Syzygium* and *Agapanthus* extracts indicated that the germination process had been initiated (Vijayaraghavan and Jain, 1984; Hodson *et al*., 1987).

The embryonic roots of *Agapanthus* treated seeds had vacuoles, lipid bodies and no proteins bodies (Figure not presented). The presence of vacuoles in the embryonic root cells of cowpea seeds treated with *Agapanthus* indicated that the cells were metabolically active and protein bodies were replaced by vacuoles (De Castro and Martinez-Hounduvilla, 1984). De Castro and Martinez-Hounduvilla (1984) further reported that proteolysis and lipolysis takes place in an active cell post imbibition and proteolysis is more rapid than lipolysis. Many lipid bodies were observed distributed within the cytoplasm of embryonic root cells of the water soaked cowpea seeds but few lipid bodies that were mostly restricted to the cell wall were observed in the embryonic root cells of seeds treated with *Agapanthus* and *Syzygium* extracts. The less number of lipids and protein bodies in the embryonic root cells of cowpea seeds treated with the plant extracts is an indication of proteolysis and lipolysis (De Castro and Martinez-Hounduvilla, 1984; Vijayaraghavan and Jain, 1984).

5.4.4 Cotyledon-embryo connecting tissue of cowpea seed treated with *Syzygium* acetone and *Agapanthus* water extracts

The cells of the cotyledon-embryo connecting tissues of cowpeas soaked in water had vacuoli and few lipid bodies along the cell wall (Fig. 5.4A).The embryonic cells of the connecting tissues of cowpea seeds treated with *Agapanthus* (Fig. 5.4B) and *Syzygium* (Fig. 5.4C) had many protein bodies and lack vacuoles. The metabolic processes were higher in the connecting tissue cells of water treated cowpea seeds than in seeds treated with *Agapanthus* and *Syzygium* extracts as evidenced by the development of vacuoles which are produced after the degradation of protein bodies. De Castro and Martinez-Honduvilla (1984) reported that the development of the organelles in imbibed seed cells is the signal of the initiation of the metabolic processes in the living cells.

This study has revealed that plant extracts seed treatment enhanced the germination process and this was evidenced by advanced ultra-structural development such as vacuoles, mitochondria with well-developed cristae, less number of lipids and protein bodies in *Syzygium* and *Agapanthus* embryonic roots and the embryo-cotyledon connecting tissue cells.

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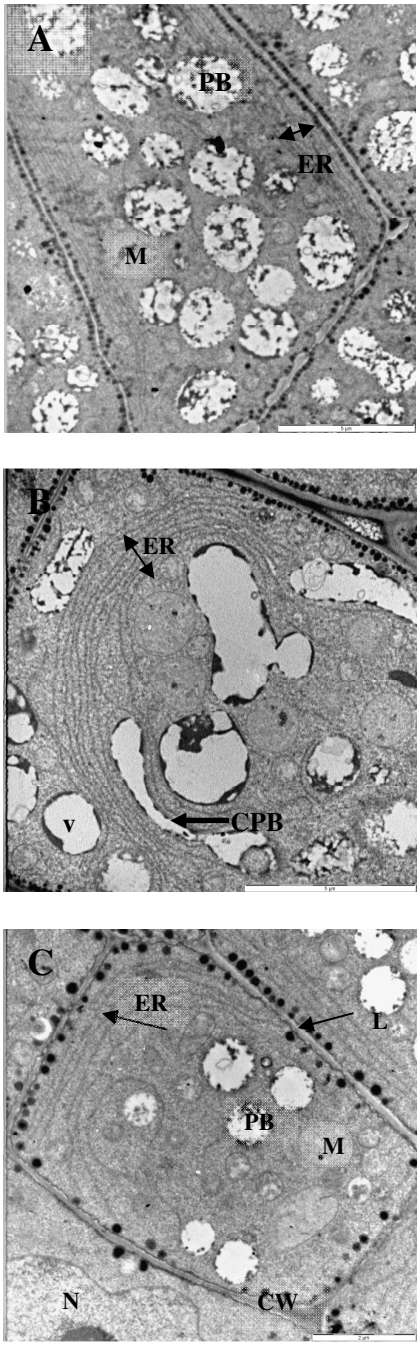

Figure 5.1 (A-C): TEM micrographs of the embryonic root of bean seed, a) treated with distilled water for 24 h, Bar = 5 µm. b), treated with 15 mg.ml^{-1} *Agapanthus* plant extract for 24 h, Bar = 5 µm and c), treated with 15 mg.ml^{-1} *Syzygium* plant extract for 24 h, Bar = 2 µm. CPB, collapsed protein body; CW, cell wall; ER, endoplasmic reticulum; M, mitochondrium; N, nucleous; L, lipids bodies; PB, protein bodies.

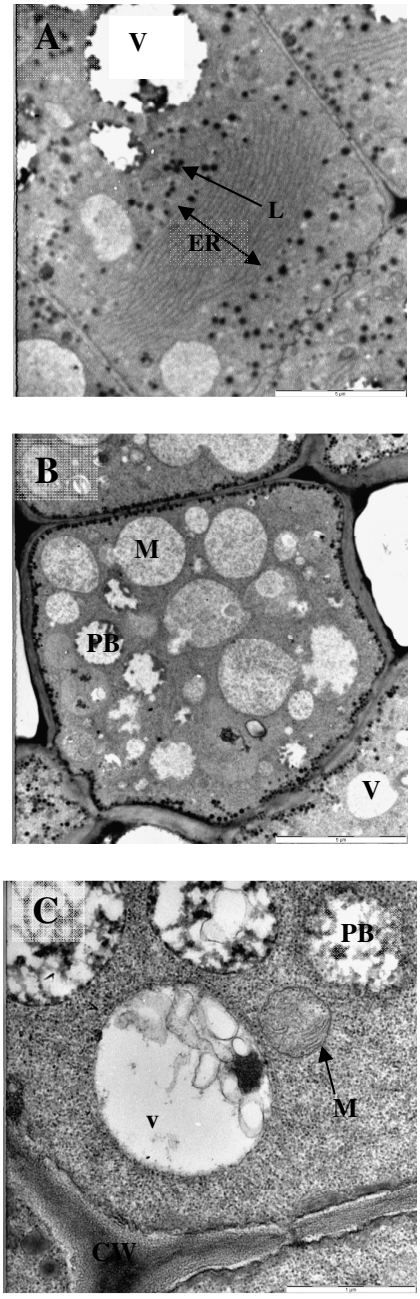

Figure 5.2 (A-C): TEM micrographs of the embryonic connecting part of bean seed, a) treated with distilled water for 24 h, Bar = 5 µm. b), treated with 15 mg.ml^{-1} *Agapanthus* plant extract for 24 h, Bar = 5 µm. and c), treated with 15 mg.ml^{-1} *Syzygium* plant extract for 24 h, Bar = 1 µm. CW, cell wall; ER, endoplasmic reticulum; M, mitochondrium; L, lipids bodies; PB, protein bodies; V, vacuole.

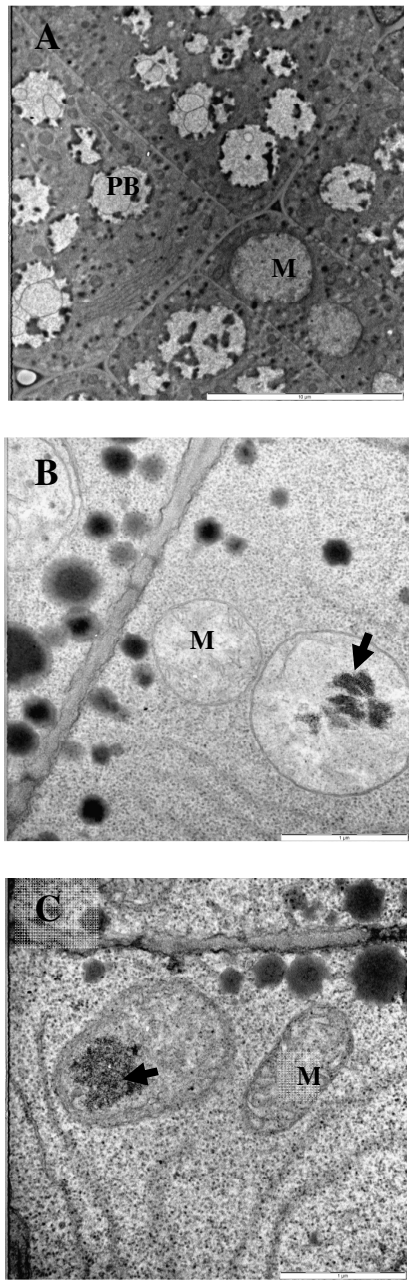

Figure 5.3 (A-C): TEM micrographs of the embryonic root of cowpea seeds, a) treated with distilled water for 24 h, Bar = 10 µm. b), treated 15 mg.ml^{-1} *Agapanthus* plant extract for 24 h, Bar = 1 µm and c), treated with 15 mg.ml^{-1} *Syzygium* plant extract for 24 h, Bar = 1 µm. M, mitochondrium; PB, protein bodies; Arrow, nucleolus.

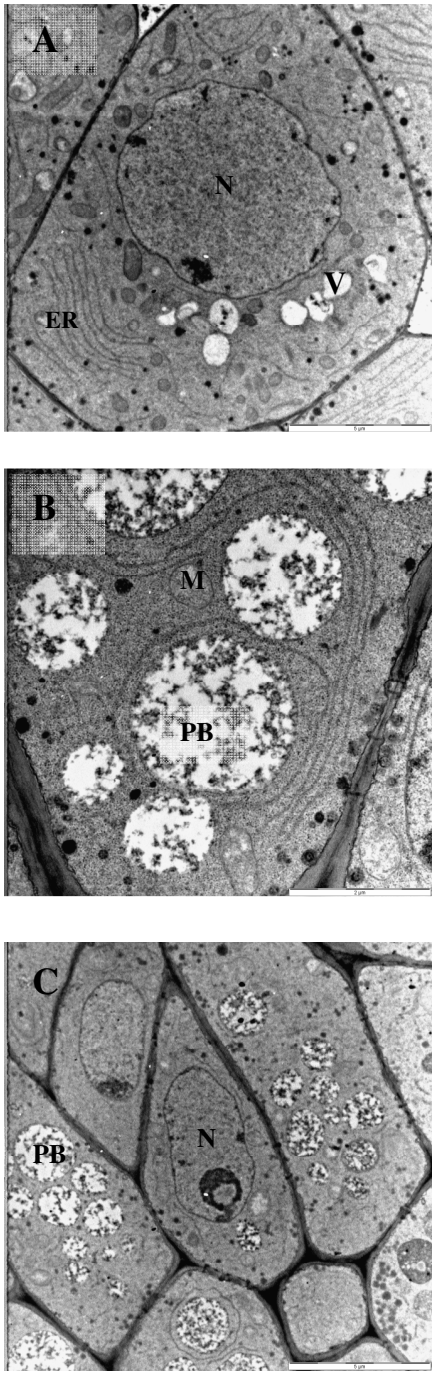

Figure 5.4 (A-C): TEM micrographs of the embryonic connecting part of cowpea seed, a) treated with distilled water for 24 h, Bar = 5 µm. b), treated with 15 mg.ml^{-1} *Agapanthus* plant extracts for 24 h, Bar = µm 2 and c), treated with 15 mg.ml^{-1} Syzygium plant extracts for 24 h, Bar = 5 µm. ER , endoplasmic reticulum; M, mitochondrium; N, nucleus; PB, protein bodies; V, vacuole.

CHAPTER SIX

EFFICACY OF FOLIAR EXTRACTS APPLICATION ON THE SEVERITY OF ANTHRACNOSE DISEASE OF BEAN (*PHASEOLUS VULGARIS* L.) AND COWPEA (*VIGNA UNGUICULATA* L. WALP)

6.1 ABSTRACT

The curative efficacy of *Agapanthus caulescens* Spreng., *Allium sativum* L., *Carica papaya* L. and *Syzygium cordatum* Hochst.ex Krauss plant extracts and the synergy of the different combinations of plant water extracts were evaluated as a spray against bean (*Phaseolus vulgaris* L.) and cowpea (*Vigna unguiculata* L. Walp) anthracnose disease. The plants were spray-inoculated with 1.3 x 10^5 spore concentrations of *Colletotrichum dematium* (Fr.) Grove var. *truncata* (cowpea) and *Colletotrichum lindemuthianum* (Sacc. & Magn.) Bri. and Cav. (bean) at primary leaf stage. The crude plant extracts (5 and 15 $mg.ml^{-1}$) were sprayed on the plants until run-off. The inoculated negative control plants were sprayed with sterile distilled water and aqueous Tween 20 solution. The synthetic fungicide (Benomyl, 3.0 $g.l^{-1}$) treated plants and non-inoculated plants represented the positive controls. The foliar application of 15 $mg.ml^{-1}$ of *Allium*, *Agapanthus*, *Carica* water extracts and acetone (5 $mg.ml^{-1}$) *Carica* extract and the combinations (2.5 $mg.ml^{-1}$ + 2.5 $mg.ml^{-1}$) of *Allium* + *Agapanthus*, *Allium* + *Carica*, *Agapanthus* + *Syzygium* and *Carica* + *Syzygium* registered low bean anthracnose (caused by *C. lindemuthianum*) disease and high leaf areas but dry weight of bean plants were statistically similar between all the treatments. These treatments can be used as alternatives to synthetic fungicides against bean anthracnose. The cowpea plants treated with 15 $mg.ml^{-1}$ water extracts of *Agapanthus* and the combinations of *Allium* + *Agapanthus*, *Agapanthus* + *Carica* and *Agapanthus* + *Syzygium* had low cowpea anthracnose

(caused by *C. dematium*) disease severity, high leaf area and dry mass. The extracts listed above have a curative effect against anthracnose diseases and are good potential foliar treatment substitutes to the synthetic fungicides.

Key words: anthracnose disease, curative effect, *Phaseolus vulgaris*, plant extracts, *Vigna unguiculata*, synergy.

6.2 INTRODUCTION

Anthracnose disease of common bean (*Phaseolus vulgaris* L.) and cowpea (*Vignaunguiculata* L. Walp.) is the main cause behind low productivity in the two crops in many smallholder farmers' fields. Anthracnose is a seed-borne disease caused by fungi of the genus *Colletotrichum.* The disease affects all the aerial parts (leaves, stems and pods) of the plants at all growth stages (Bailey and Jeger, 1992). The disease spreads by the planting of infected seeds, infected crop debris (residues) from the previous season that act as a source of inoculum and by rain splash and irrigation water. Severe losses of between 90 – 100 % can occur under high infection (Bokosi, 1986; Diaz and Lopez, 1986; Alabi, 1994; Allen *et al.*, 1996). Management of the disease is by planting pathogen free seeds and treated seed. Foliar application with fungicides is an effective way of controlling the disease in the field; however, concerns have been raised in relation to their use (Falandysz, 2000; Avenot and Michailides, 2007). Fungicides have been implicated in many human diseases such as cancer (Tangley, 1987; Wilson *et al*., 1997). Reports have indicated that residues of some fungicides have been found in vegetables and fruits posing a serious threat to the consumers as somecontain toxic chemicals (Tangley, 1987; Falandysz, 2000; Voorrips *et al.*, 2004; Shovan *et al.,* 2008). Fungicides also contaminate the environment such as soil and water thereby

affecting the ecosystem. Furthermore, the poor resource smallholder farmers fail to apply fungicides due to unavailability or high prices (Allen, 1995). There is therefore a need to search for new fungicides that are cheap, environmental friendly, relatively safe to human health and easily accessible. In the *in vitro* study (Chapter 3) different plant extracts extracted with water, acetone and ethyl acetate were evaluated for antifungal activity against the *Colletotrichum lindemuthianum* (Sacc. and Magn.) Bri. and Cav. and *Colletotrichum dematium* (Fr.) Grove var. *truncata.* The results from the above mentioned study (Chapter 3) showed that the water extracts of *Carica papaya* L. and *Syzygium cordatum* Hochst.ex Krauss were active against *C. lindemuthianum* and *S. cordatum*, *Allium sativum* L. and *Chlorophytum comosum* (Thunb.) Jacq. cv. Variegatum water extracts were active against *C. dematium*. The 5 $mg.ml^{-1}$ *C. papaya* water extract completely (100 %) inhibited *C. lindemuthianum.* The *Allium*, *Syzygium* and *Agapanthus caulescens* Spreng. acetone extracts were active against both *C. lindemuthianum* and *C. dematium in vitro* and but their efficacy as a foliar treatment to control anthracnose disease *in vivo* needs further testing.

The objectives of this study were (i) to evaluate the curative efficacy of the *A. caulescens*, *A. sativum*, *C. papaya* and *S. cordatum* plant extracts and (ii) to evaluate the synergy of the different combinations of water plant extracts as a spray on cowpea and common bean against anthracnose disease.

6.3 MATERIALS AND METHODS

The experiment was conducted in a greenhouse of the Department of Microbiology and Plant Pathology, University of Pretoria at 21 ± 5^{o}C for 7 weeks. The seeds of common bean cv. Jenny (collected from the Dry Bean Producers Organization in Pretoria) and cowpea cv. Pan 311 (sourced from Department of Agriculture, Nelspruit, Mpumalanga) were sown in 15 cm diameter pots filled with steam sterilised red loam soil at the rate of six seeds per pot.

The pots were sterilised for an hour by dipping them in aqueous 1% formalin solution prior to sowing. The seedlings were watered daily. The seedlings were spray-inoculated with a 1.3 x 10^5 spore concentration of *C. dematium* (cowpea) or *C. lindemuthianum* (bean) at primary leaf stage. The seedlings were then covered with clear polythene bags immediately after inoculation in order to maintain a high humidity. Temperatures in the glasshouse were 20±2 ^oC maximum and 17±2 ^oC minimum and the relative humidity was at 80 %. The polythene bags were removed on the fourth day after inoculation. The crude plant extracts of *Agapanthus*, *Allium*, *Carica* and *Syzygium* were sprayed onto the seedlings until run-off at 5 and 15 $mg.ml^{-1}$ concentrations after the appearance of disease symptoms on the primary leaves and stems. The combination of water plant extracts was only applied at 5 $mg.ml^{-1}$. The inoculated negative control seedlings were sprayed with sterile distilled water and aqueous Tween 20 solution. Benomyl applied at 3.0 $g.l^{-1}$ and non-inoculated plants represented the positive controls. Acetone crude plant extracts were diluted in aqueous Tween 20 solution before being applied to the seedlings. Disease evaluation was done on the sixth week post inoculation using the 1 – 9 (CIAT, 1987) anthracnose disease scoring scale as described in Chapter 4. The disease severity scores of 1 to 3 were regarded as effective whereas those of 4 to 9, not effective in controlling anthracnose disease. Leaf area was recorded using a leaf area meter and dry mass data were recorded after harvesting. The trials were repeated twice and all data were analyzed by using the Genstat 3 version (VSNi, 2008). The means were separated by the least significant difference (LSD) at a probability level of 5 %.

6.4 RESULTS AND DISCUSSION

6.4.1 Common bean anthracnose severity, leaf area and dry mass

Bean plants treated with the 15 $mg.ml^{-1}$ concentrations of *Allium* water, *Agapanthus* water, *Carica* water, 5 $mg.ml^{-1}$ concentrations of *Carica*, *Allium, Syzygium and* 15 $mg.ml^{-1}$

concentration of *Agapanthus* acetone extracts and the non-inoculated control had (P≤0.05) lower anthracnose disease severity in both the first and the second trials when compared with the negative controls (Table. 6.1).The results of 15 mg.ml^{-1}*Allium* water extract treatment agree with the findings of Curtis *et al.* (2004) that the spraying of garlic extracts post inoculation was found to reduce the rice blast disease (*Magnaporthe grisea* Hebert) Barr.) severity over the control. The foliar applied *Allium* water extract and *Carica* plant water and acetone extracts were also reported to reduce the incidence and severity of *Cercospora* leaf spot when compared to untreated crops of groundnut (*Arachis hypogaea* L.) (Bdliya and Alkali, 2008). The results of the *Carica* leaf extracts was, however, in contrast with the findings of Bautista-Banós *et al*. (2003) who found that the pawpaw fruits treated with aqueous pawpaw leaf extracts had the highest incidence and severity of *Colletotrichum gloesporioides* (Penz.) Penz and Sacc. anthracnose. The difference in results could be due to the differences in anthracnoses tested in the two studies that are also caused by different species of the *Colletotrichum* pathogen. The performance of *Agapanthus* water and acetone extracts on bean anthracnose diseases concurred with the *in vitro* results in Chapter 3.

Treatment with the water extract combinations of *Allium* + *Agapanthus*, *Carica* + *Syzygium*, *Allium* + *Carica*, *Agapanthus* + *Syzygium* resulted in low bean anthracnose disease severity that was insignificantly (P≤0.05) different from the non-inoculated control (Table. 6.1). The listed plant extracts combinations had high disease control efficacies and this could be attributed to the synergistic effect of the combination that had more antifungal effects than those of a single crude extract. Phyto-synergy normally occurs when the constituents of an extract affect different targets or interact with another constituent to improve the solubility thereby enhancing the bioavailability of one or several substances of an extract (Wagner and Ulrich-Merzenich, 2009). Therefore, synergy is claimed to be responsible for the improved effectiveness of many extracts (Wagner and Ulrich-Merzenich, 2009). Researchers reported

the synergistic effect of *A. sativum* and *C. papaya* extracts with other plant extracts against phytopathogens such as fruit rotting fungi (Sharma *et al*., 2006) and myco-pathogens of groundnuts (Ogwulumba *et al*., 2008). The efficacy of the combinations and other plant extracts against bean anthracnose corresponded to what was reported by WIPO (2008), that the combined extracts from species of the genus *Agapanthus* and *Tulbaghia violacea* Harv. showed a higher antifungal efficacy as compared to the extracts or preparations of the single species (WIPO, 2008). Benomyl treated bean plants had higher anthracnose disease severity in the second test (Table 6.1) than most of bean plants treated with extracts but lower than those of the negative controls. Amadioha and Obi (1998) reported resistance of the *C. lindemuthianum* pathogens to benomyl and resistance could be attributed to its poor performance in controlling bean anthracnose disease. The occurrence of anthracnose disease in the non-inoculated control could be due to the use of infected seeds as the disease is also spread by seeds.

Common bean plants treated with 5 $mg.ml^{-1}$ *Syzygium* water, 5 $mg.ml^{-1}$ *Carica* (both acetone and water), 15 $mg.ml^{-1}$*Allium* water, 15 $mg.ml^{-1}$ *Agapanthus* (both acetone and water) extracts, the combinations of *Agapanthus* + *Carica*, *Allium* + *Agapanthus* water extracts and all the controls had ($P \leq 0.05$) higher leaf areas (Table 6.2). An increase in plant extract concentrations increased the bean leaf area of *Allium* water and *Agapanthus* (acetone and water) extract treated plants resulted in reducing anthracnose disease and less leaf defoliation. The plants treated with the *Allium* + *Agapanthus* water extracts had the higher leaf areas of bean plants than some single (individual) extract treatment due to a synergetic effect in reducing anthracnose disease as explained above. No significant differences ($P \leq 0.05$) in terms of dry mass were observed among common bean plants treated with different plant extracts and the controls.

6.4.2 Cowpea anthracnose severity, leaf area and dry mass

All the water and acetone extracts of all the plants and all the combinations of *Allium* + *Agapanthus*, *Carica* + *Syzygium*, *Allium* + *Carica*, *Agapanthus* + *Carica* and *Agapanthus* + *Syzygium* extracts effectively reduced the severity of anthracnose disease on cowpea when compared with the inoculated control in both the first and second trials (Table. 6.3). The *Allium* extract low severity corresponded with Chowdhury *et al*. (2007) who found that spraying of *A. sativum* extract reduces the incidence of anthracnose (*C. gloeosporioides*) of mangoes (*Mangifera indica* L.) over the control. The results obtained from the cowpea plants treated with *Carica* water extract agreed with the findings of Amadioha (1998) that the leaf extract of *C. papaya* control powdery mildew in pepper (*Capsicum annum* L.). Ogwulumba *et al.* (2008) reported of the higher efficacy of the foliar applied *Carica papaya* leaf extract on the myco-pathogen of groundnut over the untreated control and their results correlated well with that of this study. Almost all the acetone plant extracts tested in this study had decreased cowpea anthracnose disease severity as concentrations increased with the exception of the *Syzygium* extract.

All the cowpea plants treated with the various plant extracts and combinations successfully reduced disease severity of cowpea anthracnose with the exception of 5 mg/ml *Carica* water extract in the first test. In the second test the *Allium* and *Syzygium* water extracts, 5 $mg.ml^{-1}$ *Syzygium* acetone and the combinations of *Carica* + *Syzygium* and *Allium* + *Carica* failed to reduce the disease severity when compared with the inoculated control (Table 6.3). WIPO (2008) reported of the higher antifungal efficacy of the combined extracts from species of the genus *Agapanthus* and *T. violacea* extracts compared to the extracts or preparations of the single species and this could be due to synergetic antifungal plant protective activities of the combinations. The WIPO (2008) report corresponded well with the results obtained from the combinations of *Agapanthus* with other extracts that elicited an

increased antifungal activity against cowpea anthracnose (*C. dematium*) as compared to the corresponding single component preparations. The combinations of *A. sativum* bulb and *C. papaya* leaf extracts with other plant extracts gave a higher antifungal activity against the cowpea anthracnose and these results concurred with the findings of Sharma *et al*. (2006) and Ogwulumba *et al*. (2008) who reported of their activities against other myco-pathogens when applied in combination with other plant extracts.

Cowpea plants treated with all the plant water extracts, *Allium* acetone and *Carica* acetone extracts, benomyl and all the combinations of extracts, except *Allium* + *Agapanthus* and the non-inoculated plants had the highest leaf area (Table 6.4). The 15 $mg.ml^{-1}$ concentration of *Syzygium* water and *Allium* (5 and 15 $mg.ml^{-1}$) acetone extracts treatments and the non-inoculated plants had the highest cowpea dry masses. The higher dry mass of cowpea could be due to high leaf area and photosynthetic material produced by healthy plants in contrast to the severely infected inoculated control. The *Colletotrichum* fungi infect the stems, petiole and the veins of the underside of the leaves on which long dark coloured lesions are caused (Pastor-Corrales and Tu, 1989) and severe infection results in leaf defoliation. The anthracnose disease interferes with the translocation of water and nutrients by causing lesions on the veins (Agrios, 2005). This results in reduced growth of the infected plant as a result of reduced photosynthesis due to the reduction in photosynthetic surface area of the plant and consequently results in reduced plant growth and low dry mass (Agrios, 2005).

The foliar application of 15 $mg.ml^{-1}$ of *Allium* water, *Agapanthus* water, 5 $mg.ml^{-1}$ *Carica* acetone and the combinations of *Allium* + *Agapanthus*, *Allium* + *Carica*, *Agapanthus* + *Syzygium* and *Carica* + *Syzygium* extract treatments registered low bean anthracnose disease and high leaf area and can be used as alternatives to the synthetic fungicides against bean anthracnose (Table 6.1). The cowpea plants treated with 15 $mg.ml^{-1}$ water extracts of

Agapanthus and the combinations of *Allium* + *Agapanthus*, *Agapanthus* + *Carica* and *Agapanthus* + *Syzygium* extracts had low cowpea anthracnose disease severity, highest leaf area and dry mass and are good potential foliar substitutes to synthetic fungicides against cowpea anthracnose. This study found that several plant extracts and extract combinations can be foliar applied to bean and cowpea plants to control anthracnose diseases without inducing any allelopathic effects on the vegetative parts of the plants. However, there is a need to determine the rate of frequency of foliar plant extract application that can effectively control the anthracnose diseases. There is also a need to assess the cost-benefit of using the above mentioned plant extracts in anthracnose disease management before recommending them to farmers.

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Table 6.1: Anthracnose disease (*Colletotrichum lindemuthianum*) severity of common bean plants treated with plant extracts.

Treatments	First test	Second test
5 mg/ml *Allium sativum (W)*	3.0 bcd	4.5 bcd
15 mg/ml *Allium sativum (W)*	1.0 a	2.6 abc
5 mg/ml *Agapanthus caulescens (W)*	3.0 bcd	4.2 abcd
15 mg/ml *Agapanthus caulescens (W)*	1.7 ab	4.2 abcd
5 mg/ml *Carica papaya (W)*	3.0 bcd	3.0 abc
15 mg/ml *Carica papaya (W)*	1.7 ab	3.8 abcd
5 mg/ml *Syzygium cordatum (W)*	3.0 bcd	4.6 bcd
15 mg/ml *Syzygium cordatum (W)*	1.0 a	5.4 cdef
5 mg/ml *Allium sativum (A)*	2.3 abc	6.2 d
15 mg/ml *Allium sativum (A)*	4.3 d	5.0 cde
5 mg/ml *Agapanthus caulescens (A)*	2.3 abc	5.0 cde
15 mg/ml *Agapanthus caulescens (A)*	2.3 abc	3.8 abcd
5 mg/ml *Carica papaya (A)*	1.0 a	3.0 abc
15 mg/ml *Carica papaya (A)*	2.3 abc	2.6 abc
5 mg/ml *Syzygium cordatum (A)*	1.0 a	3.8 abcd
15 mg/ml *Syzygium cordatum (A)*	1.0 a	6.6 def
2.5 *Allium sativum* + 2.5 *Agapanthus caulescens* (W)	1.0 a	3.4 abc
2.5 *Carica papaya* + 2.5 *Syzygium cordatum* (W)	1.7 ab	2.6 abc
2.5 *Allium sativum* + 2.5 *Carica papaya* (W)	1.7 ab	1.4 a
2.5 *Agapanthus caulescens* + 2.5 *Syzygium cordatum* (W)	1.0 a	3.0 abc
2.5 *Agapanthus caulescens* + 2.5 *Carica papaya* (W)	NT	5.4 cdef
Water (Non-inoculated)	1.0 a	1.8 ab
Inoculated control	3.7 cd	7.8 ef
Tween 20	2.3 abc	8.2 f
Benomyl	1.0 a	5.4 cdef
Significance	**	*
LSD	1.62	2.84

The figures within the same column bearing same letters are not significant at $P \leq 0.05$ or $P \geq 0.01$ level. W = water extract, A = acetone extract and NT = not tested.

Table 6.2: Mean leaf area (cm^2) and mean dry weight of common bean plants inoculated with *Colletotrichum lindemuthianum* and treated with plant extracts.

Treatment	Mean leaf area (cm^2)	Mean dry weight (g)
5 mg/ml *Allium sativum* (W)	30.8 abcdef	0.48
15 mg/ml *Allium sativum* (W)	36.8 cdefg	0.6
5 mg/ml *Agapanthus caulescens* (W)	27.7 abcdef	0.37
15 mg/ml *Agapanthus caulescens* (W)	41.8 defg	0.49
5 mg/ml *Carica papaya* (W)	45 fg	0.6
15 mg/ml *Carica papaya* (W)	31.7 bcdef	0.52
5 mg/ml *Syzygium cordatum* (W)	34.8 cdefg	0.39
15 mg/ml *Syzygium cordatum* (W)	22.9 abcd	0.36
5 mg/ml *Allium sativum* (A)	15.1 ab	0.27
15 mg/ml *Allium sativum* (A)	24.3 abcde	0.39
5 mg/ml *Agapanthus caulescens* (A)	24.8 abcde	0.57
15 mg/ml *Agapanthus caulescens* (A)	39.4 cdefg	0.59
5 mg/ml *Carica papaya* (A)	43.2 efg	0.53
15 mg/ml *Carica papaya* (A)	22.2 abc	0.35
5 mg/ml *Syzygium cordatum* (A)	32.2 bcdef	0.38
15 mg/ml *Syzygium cordatum* (A)	20.9 abc	0.32
2.5 *Allium sativa* + 2.5 *Agapanthus caulescens* (W)	34.0 bcdefg	0.62
2.5 *Carica papaya* + 2.5 *Syzygium cordatum* (W)	25.3 abdce	0.45
2.5 *Allium sativa* + 2.5 *Carica papaya* (W)	12.5 a	0.21
2.5 *Agapanthus caulescens* + 2.5 *Syzygium cordatum* (W)	26.8 abcdef	0.33
2.5 *Agapanthus caulescens* + 2.5 *Carica papaya* (W)	52.6 g	0.65
Water (Non-inoculated)	35.6 cdefg	0.49
Inoculated control	44.9 fg	0.56
Tween 20	37.3 cdefg	0.39
Benomyl	33.7 cdefg	0.5
P≤0.05	*	NS
LSD	19.13	0.29

The figures within the same column bearing same letters are not significant at P≤0.05 level. W = water extract and A = acetone extract.

Table 6.3: Anthracnose disease (*Colletotrichum dematium*) severity of cowpea plants treated with plants extracts.

Treatment	First test	Second test
5 mg/ml *Allium sativum (W)*	1.7 ab	3.4 cd
15 mg/ml *Allium sativum (W)*	3.0 abcd	3.4 cd
5 mg/ml *Agapanthus caulescens (W)*	2.3 abc	2.6 abc
15 mg/ml *Agapanthus caulescens (W)*	1.7 ab	2.6 abc
5 mg/ml *Carica papaya (W)*	4.3 cde	2.2 abc
15 mg/ml *Carica papaya (W)*	2.3 abc	2.6 abc
5 mg/ml *Syzygium cordatum (W)*	2.3 bc	3.4 cd
15 mg/ml *Syzygium cordatum (W)*	3.7 bcd	3.4 cd
5 mg/ml *Allium sativum (A)*	1.7 ab	3.8 cd
15 mg/ml *Allium sativum (A)*	3.0 abcd	2.2 abc
5 mg/ml *Agapanthus caulescens (A)*	1.7 ab	2.2 abc
15 mg/ml *Agapanthus caulescens (A)*	5.0 d	2.2 abc
5 mg/ml *Carica papaya (A)*	1.7 ab	2.2 abc
15 mg/ml *Carica papaya (A)*	2.3 abc	2.2 abc
5 mg/ml *Syzygium cordatum (A)*	1.7 ab	2.6 abc
15 mg/ml *Syzygium cordatum (A)*	2.3 abc	1.4 ab
2.5 *Allium sativum* + 2.5 *Agapanthus caulescens* (W)	1.0 a	2.6 abc
2.5 *Carica papaya* + 2.5 *Syzygium cordatum* (W)	3.0 abcd	3.0 bcd
2.5 *Allium sativum* + 2.5 *Carica papaya* (W)	2.3 abc	3.0 bcd
2.5 *Agapanthus caulescens* + 2.5 *Syzygium cordatum* (W)	2.3 abc	2.6 abc
2.5 *Agapanthus caulescens* + 2.5 *Carica papaya* (W)	NT	1.4 ab
Water (Non-inoculated)	1.0 a	1.0 a
Inoculated control	6.3 ef	4.6 d
Tween 20	7.7 f	4.6 d
Benomyl	2.3 abc	1.4 ab
Significance	**	*
LSD	2.57	1.65

The figures within the same column bearing same letters are not significant at P≤0.05 or P≥0.01 level. W = water extract, A = acetone extract and NT = not tested.

Table 6.4: Mean leaf area (cm^2) and mean dry weight of cowpea plants inoculated with *Colletotrichum dematium* and treated with plant extracts.

Treatment	Mean leaf area (cm^2)	Mean dry weight (g)
5 mg/ml *Allium sativum* (W)	47.95 abcde	0.99 abcde
15 mg/ml *Allium sativum* (W)	49.49 abcde	1.02 abcde
5 mg/ml *Agapanthus caulescens* (W)	43.97 abc	0.94 abcd
15 mg/ml *Agapanthus caulescens* (W)	57.69 e	1.12 bcdef
5 mg/ml *Carica papaya* (W)	50.25 bcde	1.02 abcde
15 mg/ml *Carica papaya* (W)	48.57 abcde	1.14 cdef
5 mg/ml *Syzygium cordatum* (W)	48.54 abcde	1.11 abcdef
15 mg/ml *Syzygium cordatum* (W)	58.52 e	1.30 fg
5 mg/ml *Allium sativum* (A)	48.16 abcde	1.52 cdefg
15 mg/ml *Allium sativum* (A)	57.03 e	1.24 cdefg
5 mg/ml *Agapanthus caulescens* (A)	40.73 ab	0.944 abcd
15 mg/ml *Agapanthus caulescens* (A)	39.23 a	0.85 a
5 mg/ml *Carica papaya* (A)	49.62 abcde	1.04 abcdef
15 mg/ml *Carica papaya* (A)	45.93 abcd	0.87 ab
5 mg/ml *Syzygium cordatum* (A)	43.95 ab	0.914 abc
15 mg/ml *Syzygium cordatum* (A)	43.58 ab	1.00 abcde
2.5 *Allium sativa* + 2.5 *Agapanthus caulescens* (W)	54.69 de	1.47 g
2.5 *Carica papaya* + 2.5 *Syzygium cordatum* (W)	45.19 abcd	1.01 abcde
2.5 *Allium sativa* + 2.5 *Carica papaya* (W)	44.41 abcd	1.06 abcdef
2.5 *Agapanthus caulescens* + 2.5 *Syzygium cordatum* (W)	51.73 cde	1.17 cdef
2.5 *Agapanthus caulescens* + 2.5 *Carica papaya* (W)	42.40 adc	1.029 abcde
Water (Non-inoculated)	56.85 e	1.23 ef
Inoculated control	49.12 abcde	1.17 cdef
Tween 20	49.97 bcde	1.19 def
Benomyl	50.41 bcde	1.17 cdef
P≤0.05	*	*
LSD	10.60	0.27

The figures within the same column bearing same letters are not significant at P≤0.05 level. W = water extract and A = acetone extract

CHAPTER SEVEN

GENERAL DISCUSSION

The improvement of food security and income of smallholder farmers by reducing legume disease incidences and severity through the use of effective botanicals as an alternative to synthetic fungicides is a new direction that scientists have taken. The studies described herein were undertaken to screen a range of plant extracts *in vitro* and *in vivo* as seed and foliar treatments for the control of anthracnose disease of common bean (*Phaseolus vulgaris* L.) and cowpea (*Vigna unguiculata* L. Walp). A study was also conducted on the ultra-structural changes taking place within the common bean and cowpea seed cells after plant crude extract treatment and the effects of these treatments on common bean and cowpea seed germination and emergence. The efficacies of the combinations of different plant water extracts in the control of anthracnose diseases were also investigated.

In the *in vitro* study (Chapter 3), the complete (100 %) inhibition of *Colletotrichum lindemuthianum* (Sacc. and Magn.) Bri. and Cav. and *C. dematium* (Fr.) Grove var. *truncata* in the agar infusion technique was observed in *Allium* acetone and ethyl acetate extracts which gave 0.78 and 3.13 $mg.ml^{-1}$ as MIC in the microtitre dilution technique. The *Allium* water extract yielded a MIC of 6.25 $mg.ml^{-1}$ on *C. dematium* and this result agreed with that of Shovan *et al.* (2008) who reported antifungal activity of *Allium* water extract on the growth of *C. dematium*. *Carica* 5 $mg.ml^{-1}$ leaf water extract compared well with the commercial fungicide, Celest® XL, by effectively inhibiting the growth of *C. lindemuthianum* by 100 % in the agar infusion technique. The differences in chemical or genetic composition between the two *Colletotrichum* species used in this study could be responsible for the failure of the *Carica* water extract to inhibit the growth of *C. dematium*. The inhibitory activity among the

solvent extracts used in the *in vitro* study showed that acetone plant extracts performed best and this concurred with the findings of Masoko *et al.* (2005) who found that acetone extracts were superior to other extraction solvents such as hexane, dichloromethane and methanol. Masoko *et al*. (2005) speculated that water fails to extract non-polar active compounds in plant materials and this could be the reason why most plant water extracts under-performed in inhibiting the fungi.

The seed treatment study (Chapter 4) showed that *Allium* acetone and *Syzygium* water plant extracts improved the germination of IT93k5132 cowpea seeds compared to DMSO and the water treated control. *Allium sativum* extract was reported to improve the germination of tomato (*Lycopersicum esculentum* L.), chilli (*Capsicum annuum* L.) and jowar (*Sorghum bicolor* L.) seeds (Karade *et al*., 2010) and *Agapanthus* extract increased germination of radish (*Raphanus sativus* L.) seeds compared to the water control (WIPO, 2007). The lower emergence percentages observed in PAN 311 cowpea seeds treated with the higher concentration (15 $mg.ml^{-1}$) of *Agapanthus* extracts (both water and acetone) could be explained by the fact that low concentrations of plant extracts may promote seed germination whilst high concentrations may decrease germination (WIPO, 2007). Reduced vigour due to long seed storage period could be the reason for low emergence percentages of 2009 bean and IT93K5132 cowpea seeds because they were from the 2009 production season unlike PAN 311 and bean (2010) seed that were from the 2010 harvest and gave higher emergence percentages. A general decrease in emergence percentage and increase in MET of both crops as some plant extract concentrations increased (from 5 to 15 $mg{\cdot}ml^{-1}$) in most treatments was observed in the study and could be attributed to toxicity of the extracts. The bean and cowpea plants grown from *Syzygium* (both water and acetone) extracts treated seeds had lower cowpea and bean anthracnose disease incidence and severity when compared with the negative control. The *in vivo* performance of *Syzygium* extract was in agreement with results

from the microtitre dilution assay (Chapter 3) where it was active against both *C. dematium* and *C. lindemuthianum.* Acetone plant extracts showed the highest inhibitory activity among the other solvent extracts *in vitro* and also gave good result *in vivo* against *C. lindemuthianum*. Superiority of acetone extracts over other extraction solvents agreed with Masoko *et al.* (2005) who found that it was a better extracting solvent than other organic solvents and water. The *in vivo Allium* water extracts results concurred with the *in vitro* findings and also with the results of Shovan *et al.* (2008) where garlic water extracts inhibited the growth of *C. dematium*.

In Chapter 5, the embryonic roots from *Syzygium* extract treated seeds had few lipid bodies along the cell wall whereas the control and *Agapanthus* extract treated seed had more lipid bodies restricted to the cell wall. This is an indication that germination had already started in the *Syzygium* extract treated seeds. Hodson and co-workers (1987) reported that the layer of lipid bodies gradually disappears from near the plasma membrane and becomes incorporated into the plasma membrane during germination. The rate of lipid transformation is proportional to seeds germination rate (Mollenhauer and Totten, 1971) meaning that the germination rate of the *Syzygium* treated seeds could have been higher than that of the *Agapanthus* treated seeds and the control. In cowpea, the cells of connecting parts of the embryo and cotyledons of seeds treated with the *Agapanthus* water extract had more endoplasmic reticulum than those treated with the *Syzygium* acetone extract and the untreated control. Fernández de Castro and Martinez-Honduvilla (1984) reported that the development of the organelles in imbibing seed cells is the signal of the initiation of the metabolic processes in the living cells. More endoplasmic reticulum in *Agapanthus* treated cowpea seeds could have contributed to the low MET observed in Chapter 4. The coalescence of some of the protein bodies observed in the embryonic cells of bean radicles is a sign of damage that could be due to plant extract toxicity.

In Chapter 6, low severity of bean anthracnose disease was registered in plants treated with *Allium* 15 mg.ml^{-1} water extract whereas in cowpea low anthracnose severity was observed in plants treated with *Allium* 15 mg.ml^{-1} acetone extracts. Treatments with the high (15 mg.ml^{-1}) concentration of *Carica* leaf water extracts and 5 and 15 mg.ml^{-1} acetone extract had low bean and cowpea anthracnose severities when compared with the inoculated controls. The efficacy of the foliar applied *Allium* and *Carica* plant extracts in reducing the incidence and severity of *Cercospora* leaf spot and rice blast crops of groundnut (*Arachis hypogaea* L.) has been reported (Curtis *et al.*, 2004; Bdliya and Alkali, 2008). Results obtained in common bean and cowpea plants treated with *Carica* leaf extract in the present study were in contrast with the findings of Bautista-Banós *et al*. (2003) who reported that pawpaw (*Carica papaya* L.) fruits treated with aqueous pawpaw leaf extracts had the highest incidence and severity of *Colletotrichum gloeosporioides* (Penz.) Penz and Sacc. anthracnose. However, this anthracnose and the two anthracnoses in the present study differ in the plant tissues that they infect and are caused by different species of *Colletotrichum* which may account for the different results obtained in the two studies. The activity of *Agapanthus* water and acetone extracts against bean and cowpea anthracnose diseases concurred with the *in vitro* results in Chapter 3. An increase in extract concentration increased toxicity of the extracts to *C. lindemuthianum* hence the reduced bean disease severity at higher concentrations. The combination of *Allium* + *Agapanthus*, *Carica* + *Syzygium*, *Allium* + *Carica* and *Agapanthus* + *Syzygium* water extracts effectively reduced bean anthracnose disease and *Allium* + *Agapanthus*, *Agapanthus* + *Carica* and *Agapanthus* + *Syzygium* water extracts reduced the severity of cowpea anthracnose disease. This could be attributed to the synergistic effects of the combination that had more antifungal effects than those of a single crude extract. Wagner and Ulrich-Merzenich (2009) stated that the synergy of many extracts is responsible for the improved effectiveness of extracts. This statement was

supported by the findings of Sharma *et al*. (2006) who showed the synergistic effect of *A. sativum* and *Azadirachta indica* A. Juss extracts in reducing the sporulation of some fruit rotting fungi. Ogwulumba *et al*. (2008) also found that disease incidence of myco-pathogens of groundnuts treated with *C. papaya* leaf and bitter leaf extracts in a pre-soak and after post-germination spraying was more effective than the preparation of the single species extracts. The present study also reveals that combinations of *Agapanthus* and other plant extracts had a higher efficacy against bean and cowpea anthracnose concurring with the WIPO (2008) report that explained the higher antifungal efficacy elicited by the combination of *Agapanthus* and *Tulbaghia violacea* L. extracts as compared to single preparation of the extracts. Leaf defoliation occurs under severe anthracnose of bean and cowpea which attacks all the vegetative parts including the pods (Pastor-Corrales and Tu, 1989). Low leaf defoliation was observed in all cowpea and bean plants that recorded low anthracnose disease severity which in turn resulted in high leaf area. The *Allium* + *Agapanthus* extract combination treatment had the highest cowpea dry mass. Cowpea plants treated with all the combinations of plant extracts had low disease severities such that photosynthetic process of the plants were high compared to the inoculated control due to the higher leaf area they possessed. The *Colletotrichum* fungi infect the stems, petiole and the veins of the underside of the leaves on which long dark coloured lesions are caused (Tu, 1988; del Rio and Bradley, 2002). These lesions and defoliation of leaves due to anthracnose disease reduces the photosynthetic surface of the plant consequently resulting in reduced plant growth and low dry mass (Agrios, 2005).

This study revealed that *A. sativum*, *Agapanthus*, *C. papaya* and *S. cordatum* plant extracts have antifungal activities and can be used as alternative seed treatments and foliar fungicides against the anthracnose diseases of legumes (cowpea and common bean) instead of the synthetic fungicides without causing any negative effect on seed germination,

emergence, ultra-structure of seeds and plant growth. There are still, however, some grey areas in which further research is required before the plant extracts used in this study can be recommended to the farmers. The testing of the antifungal activities of the plant extracts used in this study against different races of *C. lindemuthianum* and *C. dematium* need to be evaluated. The effect of the soaking period of legume seeds in plant extracts for different lengths of time is needed in order to estimate the appropriate length of time the seeds can be soaked to effectively control anthracnose disease without causing seed damage. Furthermore, the frequency of plant extracts foliar application and economic analysis of using plant extracts in controlling anthracnose also need to be evaluated.

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