Evaluation of fungicide seed treatments to control seedling diseases of cowpea

by

Daniel Mkhathazi Khumalo

Submitted in partial fulfilment of the requirements for the degree of

M.Inst. Agrar. (Plant Protection)

In the Faculty of Natural and Agricultural Sciences
Department of Microbiology and Plant Pathology

University of Pretoria

Pretoria

October 2006

DECLARATION

I declare that the dissertation herewith submitted for the degree of M.Inst. Agrar (Plant Protection) at the University of Pretoria, has not been submitted by me for any degree at any other university or institution of higher education

……..

Daniel Mkhathazi Khumalo

ACKNOWLEDGEMENTS

The author would like to thank the following people who made valuable contributions to the completion of this thesis:

My supervisor Prof. T.A.S. Aveling and co-supervisor Dr. Q. Kritzinger for their valuable criticism, scientific advice, support and encouragement.

My deep gratitude goes to my family for their continuous and valuable support during the hard times of my studies.

The people in the department of Microbiology and Plant Pathology, especially Zelda and Veloshinie for their valuable criticism.

All the people in lab 2-12 for their support and encouragement.

All friends who supported me during the progress of this research and my stay at the University of Pretoria.

The almighty God for giving me strength to complete this thesis

LIST OF TABLES

LIST OF FIGURES

SUMMARY

Cowpea is an important food crop and is increasingly being cultivated by small-scale farmers in South Africa. Cowpea is susceptible to a wide range of seedborne diseases, which causes damage to the crop at all stages. Seedling diseases caused by pathogens like *Rhizoctonia solani* (Kuhn), *Pythium ultimum* (Trow) and *Fusarium solani* (Mart) App. and Wol attack cowpea, and result in low yields especially in rural areas where little or no control measures are taken against these pathogens.

Different concentrations of Celest® XL [fludioxonil (25gai/ L) and mefenoxam (10gai/L)] were evaluated against these pathogens and their effect on germination. Thiram (500gai/L DS) was used as a standard fungicide. In the *in vitro* assay, media was amended with Celest® XL at 1x (0.06ml), 1.25x (0.75ml) and 2x (0.12ml) the recommended rate. Growth diameter was measured on day 3, 6, and 9. All treatments significantly inhibited mycelial growth of *P. ultimum, F. solani* and *R. solani* when compared to the control.

A germination test was performed according to the rules of the International Seed Testing Association (ISTA) (2005). Celest® XL improved cowpea germination, and increased shoot and root length. Disease incidence was significantly lowered by all the treatments when compared to the control. In greenhouse trials, seedling trays were filled with pasteurised growing medium and assigned randomly with four replications per treatment. Each replication consisted of 25 plants. The growing media was artificially inoculated with each of the three pathogens by placing two mycelial plugs in each cell of the seedling trays. Fungicides were applied as a seed slurry treatment at a concentration of 0.6g/500g thiram and 1x (100ml/100kg seed), 1.25x (125ml/100kg seed) and 2x the recommended rate (200ml/100kg seed) Celest® XL and mixed for 5min. The control was treated with water using the same procedure.

It was found that all the treatments significantly inhibited mycelial growth of all three pathogens in the *in vitro* test. Germination performance was enhanced by treating the cowpea seeds with thiram and Celest® XL. The results also showed that percentage emergence was increased by all treatments when compared to the control. All the

treatments, significantly reduced disease incidence on cowpea seedlings in the greenhouse. All the treatments when compared to the inoculated control, significantly increased plant height and dry shoot mass.

This study provides sufficient data to warrant further testing of the treatments under field conditions. The residual effects of Celest® XL and thiram also needs to be determined before the fungicides can be registered as seed treatments of cowpea in South Africa.

CHAPTER 1

GENERAL INTRODUCTION

Cowpea *(Vigna unguiculata* [L.] Walp.) is the most important legume grown in the tropical savanna zones of Africa (Singh *et al*., 1997). Although indigenous to southeastern Africa, cowpea has spread worldwide and is extensively cultivated and consumed in regions of Asia, South and Central America, the Caribbean, the United States, the Middle East and southern Europe (Singh *et al*., 1997). Cowpea is a preferred staple food in many regions of Africa (Cesse, 1995). Its desirable effects are reflected in the fact that leaves, immature pods, fresh seeds (green pods) and dry grain can be eaten or marketed (Cesse, 1995).

According to Pendulosi (1997), some varieties have a short life cycle and are able to provide food during the hunger period. This is the period at the end of the wet season when food can become extremely scarce in semi-arid regions of sub-Saharan Africa (Aykoyd *et al*., 1982). The dry grain is also commonly milled and consumed in numerous traditional main dishes of Africa as porridge and bread, fed to young children as weaning food and eaten as processed snack foods (Pendulosi, 1997).

The mature grain contains 23-25% protein, 50-67% starch, vitamins B such as folic acid, which are important in preventing birth defects and essential micronutrients such as iron, calcium and zinc (Kitch *et al*., 1998). Although a significant amount of cowpea is commercialized, it plays a critical subsistence role in diets of many households in Africa, providing nutrition not obtained from cereals (Cesse, 1995).

According to Sinclair (1993), cowpea is susceptible to a number of diseases, which result in great losses in crop yield from germination to harvesting. The major disease problem of cowpea in most areas results from infection with soilborne pathogens (Emechebe & Florini, 1997). Root rot and damping-off are diseases that have been shown to play a significant role in reducing cowpea yields. Symptoms vary and include rapid death of

young succulent plants, discolouration of taproots, longitudinal cracks of the stems, stunting, wilting and poor yields (Langyintiuo *et al.*, 2003).

Complete control of root rot and damping-off is difficult, and no variety of cowpea is resistant to root rot (Emechebe & Florini, 1997). Persistent suitable weather for damping-off prior to development of the first true leaf and also crowding of seedlings due to poor seed spacing may increase damping-off (Cesse, 1995).

1.1 Motivation for the study

The damping-off and stem rot diseases syndrome caused by *Rhizoctonia solani* (Kühn), *Pythium ultimum* (Trow) and *Fusarium solani* (Mart) App. and Wol. are harmful to many cultivated crops including cowpea (Cesse, 1995). These diseases result in poor stands due to seedling infection by the fungus after seed has germinated but before the seedling has emerged above soil line (Latude-Dada *et al.*, 1993). In West Africa yield losses due to damping off diseases is estimated to be up to 60% (Latude-Dada *et al.*, 1993). Kassou *et al.* (2001) reported that in Benin loss due to seedling diseases is estimated to over 44%. Economic losses due to these diseases have not yet been determined in southern Africa. These diseases result in reduced yields due to poor germination. Cowpea is also susceptible to most diseases that attack legumes including leaf and pod diseases (Singh *et al.*, 1997). Treating the seed before planting is one method to control the seedling diseases of cowpea. In South Africa there is presently no chemical registered for the control of seed and seedling diseases on cowpea. According to Smith (1997), treating cowpea with fludioxonil and thiram was effective in controlling some cowpea diseases like *Colletotrichum dematium* (Pers) Grove.

1.2 Aim of the study

The aim of this study was to evaluate the efficacy of different concentrations of Celest® XL [fludioxonil (25gai/L) and mefenoxam (10gai/L)] and thiram (500gai/kg DS) in controlling three pathogens that causes root rot and damping-off of cowpea seedlings namely, *R. solani* (Kühn), *P. ultimum* and *F. solani*.

1.2.1 Objectives

a) To test the efficacy of Celest® XL and thiram in controlling *R. solani*, *F. solani* and *P. ultimum* under laboratory conditions (*in vitro* test).
b) To determine the effect of Celest® XL and thiram on seed germination, shoot length, root length, percentage abnormal and diseased seeds.
c) Evaluate different concentrations of Celest® XL and thiram as slurry seed treatments in the greenhouse for control of *R. solani*, *F. solani* and *P. ultimum* on cowpea seedlings.

1.3 Structure of thesis

Chapter two. Literature review. A brief discussion of the host plant, its taxonomy and uses and the fungal diseases that affect cowpea is given. A more detailed review of *R. solani*, *F. solani* and *P. ultimum* on cowpea and how these pathogens have been controlled using chemical seed treatment is then presented.

Chapter three. This chapter evaluates the efficacy of Celest® XL and thiram against *R. solani*, *F. solani* and *P. ultimum* under laboratory conditions (*in vitro* test).

Chapter four. In this chapter the effect of the fungicides (Celest® XL and thiram) on cowpea seed germination, shoot length, root length, percentage abnormal and diseased seeds are reported. Seeds were treated with thiram and different concentrations of Celest® XL. A germination test was then conducted according to the International Seed Testing Association (ISTA) rules.

Chapter five. This chapter reports on the evaluation of different concentrations of Celest® XL and thiram as slurry seed treatments for controlling *R. solani*, *F. solani* and *P. ultimum* on cowpea seedlings in the greenhouse. Percentage emergence, diseased seedlings, plant height and shoot and root dry mass were determined.

Chapter six. A general discussion is given on the results obtained in this study.

CHAPTER 2
LITERATURE REVIEW

2.1 The host: Cowpea (*Vigna unguiculata* [L.] Walp.)

2.1.1 Introduction

Cowpea (*Vigna unguiculata* [L.] Walp.) is a tropical legume of African origin (Devries & Toenniessen, 2001) and is distributed worldwide, especially in semi arid and arid areas (Ng, 1995). The legume plays a significant role in the livelihood of poor people in underdeveloped countries because it is utilized as a food crop, cash crop and for animal feed (Singh *et al*., 1997).

Cowpea is a pulse crop used by most West and Central African farmers. It is in fact a key staple for the poorest sector of many developing countries (Langyintiuo *et al*., 2003). Cowpea is also considered a significant component in the diets of people in developing countries of Africa, Latin America and Asia where it is used as a dietary protein to complement cereals (Phillips *et al*., 2003). Due to its lower grain yield, cowpea is considered as a minor crop. This has resulted in scarcity of cowpea production data worldwide and hence it is not significant in world trade (Singh *et al*., 1997).

According to Singh & Eaglesfield (2000), cowpea can be grown under various production systems including rain fed and irrigated as well as in areas of poor soils in low rainfall regions (Ng, 1995). Cowpea is an extremely resilient crop, cultivated under some of the most extreme agricultural conditions in the world (Amin, 1991). Cowpea varieties grown in the Sahel and on the fringes of the Sahara are drought and heat tolerant. Other cultivars are tolerant to acidic soils, extremely poor soil fertility, and shading from other crops (Summerfield & Roberts, 1985).

The high diverse plant architecture of cowpea has allowed farmers to develop varieties which fill a wide range of unique niches; highly determinate varieties are grown for grain

in monoculture situations, while spreading types are grown as dual-purpose (grain/ fodder) crop interplanted with cereals, and as a relay crop using residual moisture (Phillips *et al.*, 2003). Cowpea can be intercropped with sorghum (*Sorghum bicolor L.*), maize (*Zea mays* L.), groundnuts (*Arachis hypogaea* L.) and millet (*Eleusine coracana* L.) (Devries & Toenniessen, 2001).

According to Cesse (1995), cowpea is remarkably susceptible to a wide range of pests and pathogens, which can cause damage to the crop at all stages of growth. Viruses, fungi, nematodes and parasitic flowering plants induce diseases of cowpea (Hapton *et al.*, 1997).

2.1.2 Taxonomy, origin and distribution

Cowpea is a dicotyledonous plant belonging to the order Fabales, family Fabaceae, sub-family Faboiddea, tribe Phaseoleae, sub tribe Phaseolinae and genus *Vigna* (Singh *et al.*, 1997). According to Allen (1983), the genus *Vigna* comprises some 160 species; most from African origin. Only seven species are cultivated and five of these are of Asian origin. Hall (1997) mentioned that five subspecies of *Vigna unguiculata* are recognized. These are *unguiculata*, the cowpea; *cylindrical,* the catjana and *sesquipedalis,* the yard long or asparagus bean; *dekindtiana* and *meuseusis*.

All evidence shows that cowpea originated in Africa, although the location where the crop was first domesticated is uncertain. Species *unguiculata* is thought to be a West African Neolithic domesticate whose progenitors were the wild weed species *dekindtiana* and *meusensis* (Kitch *et al.*, 1998). According to Sithole-Niang (2000), there is little archeological evidence to support the West and Central Africa origin of cowpea. This was because of the lack of major civilization in the sub-Sahara part of Africa. Pendulosi *et al*. (1997) reported that southern Africa is the center of genetic diversity because the most primitive of wild cowpea occurs in Namibia from the west, across Botswana, Zambia, Zimbabwe and Mozambique to the east, and the Republic of South Africa and Swaziland to the south.

According to Sithole-Niang (2000), cowpea cultivation in west and central Africa covers more than eight million hectares. Nigeria is the largest producer followed by Niger, Brazil and India which are also main centers of production of dry cowpea seed (Quass, 1995).

Cowpea was introduced from Africa to India between 2000 and 3500 years ago, probably at the same time as the introduction of sorghum and millet. In India subspecies *cylindrica* evolved from *unguiculata* and *sesquipedalis* (Hall, 1997). Cowpea reached Europe from Asia and perhaps from North Africa before 300BC, and the Spanish took the crop to the West Indies in the seventeenth century AD. Most cultivars reached the new world from West Africa with the slave trade, reaching southern USA early in the eighteenth century (Kitch *et al.*, 1998).

Cowpea is now distributed throughout the tropics and subtropics. This is due to the fact that cowpea grows successfully on a wide range of soils (Cesse, 1995). For a better crop the soil must be well drained. Cowpea is sensitive to water logging as it reduces its ability to fix nitrogen (Tindal, 1983). Cowpea is a crop species but the variety requirements in terms of plant type, maturity seed type, colour preference and use pattern are extremely diverse from region to region (Singh *et al.*, 1997).

2.1.3 Uses of cowpea

Cowpea is of major importance to the livelihood of millions of relatively poor people in less developed countries of the tropics. From production of this crop, rural families derive food, animal feed and cash together with spillover benefits for their farmlands. Cowpea is widely traded out of the major production areas and provides a cheap, nutritious food for relatively poor urban communities (Singh *et al.*, 1997).

Like other food legumes, cowpea has a high quality protein content. Cowpea is served as a natural protein to supplement other food crops (Hapton *et al.*, 1997). It is often called the meat for the poor people, since this protein is cheaper than other protein sources (Atachi *et al.*, 1984). Its grain contains approximately 24.8% protein and 64%

carbohydrates and other nutrients needed by the body, as shown in Table 2.1 (Singh & Rachie, 1985).

Table 2.1 Percentage nutrient content of mature cowpea seeds (Singh & Rachie, 1985)

Protein	24.8%
Fat	1.9%
Fiber	6.3%
Carbohydrate	63.6%
Thiamine	0.00074%
Riboflavin	0.00042%
Niacin	0.00281%

According to Aykoyd *et al.* (1982), one of the most important nutritional characteristics of food legumes including cowpea, is that they complement cereal grains. The protein quality is synergistically improved in cereal-legume mixes because of lysine contributed by the cowpea and the methionine contributed by the cereals (Singh *et al*., 1997).

In its fresh form young cowpea leaves, immature pods and peas are used as vegetables, in west and southern Africa. Several snacks and main meal dishes are prepared from the grain (Singh *et al*., 1997). Cowpea paste, which is prepared from dried peas, is the primary ingredient for the well-known Nigerian fried product, akara (Bulgarelli *et al*., 1988). In Asia, green pods are eaten whereas in east and southern Africa the tender leaves are regularly picked and eaten like spinach (Singh *et al*., 1997). In Africa, the young leaves are sometimes dried for use in soups, while the haulms are fed to livestock. In advanced agriculture, cowpeas are used mostly for fodder and as a cover crop, though Black eye types are grown for dry seed on a large scale in California (Allen, 1983).

Some cultivars provide a fibre obtained from the peduncles (Kitch *et al.,* 1998). The fibre of cowpea is used to make fishing lines and has also been considered as a source of pulp to make good quality paper (Summerfield & Roberts, 1985). Emechebe & Florini (1997) stated that the agronomical importance of cowpea cultivars with spreading

interdeterminate or semi arid determinate bushy growth provides ground cover, hence suppressing weeds and providing some protection against soil erosion (Singh & Rachie, 1985).

According to Maude (1996), cowpeas like other legumes can symbiose with nodular bacteria (rhizobia) present in most if not all-tropical soils. Biological nitrogen fixation is beneficial to subsequent cereal crops in rotation or in association with cowpea in intercropping (Maude, 1996). Maximum nitrogen fixation is an economical way to deal with the shortage of expensive nitrogenous fertilizers in tropical countries (Singh *et al.*, 1997).

2.1.4 Cowpea production constraints

Cowpea is attacked by over 35 disease-causing viruses, bacteria, fungi and nematodes (Singh *et al.,* 1997), some of which cause significant reductions in yield.

2.1.4.1 Fungal diseases

2.1.4.1.1 *Pythium* soft stem rot

Pythium spp. are considered significant in warm, humid tropical conditions such as those of the rainforest, the southern part of Southern Guinea, the savannah of west and central Africa and humid, subtropical zones of India, because of the damage they cause to crops (Singh *et al*., 1997).

Pythium soft stem rot is characterised by a grey-green, water-soaked girdle of the stem extending from the soil and including the lower branches (Singh & Rachie 1985; Davis, *et al*., 1997). According to Singh *et al*. (1997), the slimy stem base is covered by white, cottony mycelial growth during periods of high humidity. The disease incidence is increased with high plant populations, while use of average plant populations can lower the infection rate (Singh *et al.,* 1997). Some fungicides give better disease control when used as a seed treatment rather than a soil drench (Singh *et al*., 1997).

2.1.4.1.2 *Fusarium* wilt

Symptoms of *Fusarium* wilt (caused by *Fusarium oxysporum* Schl. f.sp. *tracheiphilum* (E.F. Smith) Snyder and Hansen) include stunting of the affected cowpea plant, chlorosis, dropping, premature defoliation, withering of leaves and brownish purple discolouration of vascular tissues (Singh & Rachie, 1985; Davis *et al.,* 1997; Boyhan *et al*., 1999). The leaves become flaccid and chlorotic, and young plants show fairly rapid wilting leading to death. Transmission occurs through soil and probably seed (Singh *et al*., 1997).

The disease can be prevented by using resistant cowpea varieties (Singh & Rachie, 1985). Root knot nematodes provide conducive conditions for the pathogen to infect the plant, therefore their control will help in reducing the rate of infection by *Fusarium* (Davis *et al*., 1991).

2.1.4.1.3 *Rhizoctonia* diseases

Rhizoctonia solani Kühn is a soilborne pathogen that causes stem canker, storage rot, aerial blight, and seedling damping-off diseases in many crops such as cowpea, soybean, carrots (*Daucus carota* L.) and potato (*Solanum tuberosum* L.) (Carisse *et al*., 2001). *Rhizoctonia bataticola* [(Taub.) Butler] causes a seedling disease of cowpea that is commonly known as charcoal rot. The pathogen overwinters as sclerotia under adverse soil environmental conditions (Carisse *et al*., 2001).

Some fungicides presently registered for control of *Rhizoctonia* diseases are chloroneb, tebuconazole, fludioxonil, metalaxyl, carboxin and thiram (McMullen & Bradley, 2005). Use of biological control agents such as endophytic bacteria and fungal antagonists including *Trichoderma* spp. and *Gliocladium virens* Miller, Giddens and Foster have also shown potential for practical applications in agriculture (Carisse *et al*., 2001).

2.1.4.1.4. Anthracnose

Anthracnose of cowpea is caused by the pathogen *Colletotrichum gloeosporioides* f.sp. *aeschynomene* (CGA) (Singh *et al*., 1997). The pathogen attacks the stem, leaves and pods (Boyhan *et al*., 1999). Symptoms are brown, sunken and lenticular lesions that expand quickly and coalesce to girdle stems, peduncles and petioles on susceptible species of cowpea (Allen, 1983; Valenzuela & Smith, 2002). The primary source of inoculum is seed and secondary sources are rain-splash, air currents and contact with man and animals (Singh & Rachie, 1985). Wet and humid conditions during the growing season are favourable for anthracnose (Singh *et al*., 1997). The severity of the disease can also be increased by high plant populations (Edema *et al*., 1997).

According to Singh *et al*. (1997), the use of resistant varieties controls anthracnose diseases. Use of foliar fungicides such as benomyl and carbendazim can reduce epidemics by 40 to 45% (Singh *et al*., 1997). Some strains of *Colletotrichum* species with resistance to fungicides such as carbendazim and thiophanate-methyl have been discovered in India (Singh *et al*., 1997). Alcohol and water extracts of *Piper nigrum* L. Query IPNI, *Ocimum sanctum* L. and *Citrus limon* L. Burm are considered to be effective in reducing diseases of *Colletotrichum* spp. of cowpea *in vitro* and *in vivo* (Amadioha, 2003).

2.1.4.1.5 *Colletotrichum dematium*

Colletotrichum dematium is a soilborne pathogen that was first reported on cowpea in South Africa by Smith *et al*. (1999). The pathogen survives in a wide range of temperatures and tropical hosts, many of them being legumes (Smith, 1997). According to Alabi & Emechebe (1990), *C. dematium* was found not only to be a saprophyte but it is also associated with irregular pale to dark brown lesions on a variety of hosts in different plant families.

The fungus overwinters in plant debris and seeds. Its causes pre- and post-emergence blight or death of seedlings (Roy, 1982). Seedlings developing from infected seeds show stunting of cotyledons, which often fail to open, as the testa remains attached to them.

Lesions on cotyledons are initially discrete, but later coalesce leading to necrosis and sometimes dehiscence of cotyledons (Roy, 1982). Advancement of the fungus downwards from the cotyledons leads to girdling of the young stems and eventual death of seedlings (Roy, 1982). The fungus also causes lesions on hypocotyls at points above and below the soil line, which are related to the internal spread of the pathogen from infected cotyledons. *Colletotrichum dematium* is controlled by mancozeb, imazalil, fludioxinil and thiram on cowpea (Smith *et al.*, 1999).

2.1.4.1.6 Ascochyta blight

Ascochyta phaseolorum Sacc. causes a seedborne disease in cowpea (Allen, 1983). Symptoms are severe defoliation and lesions on the stems and pods, which can result in death (Singh *et al.*, 1997). The pathogen overwinters in infected debris and in certain perennial hosts (Allen, 1983). Primary inoculum is seed and plant debris and secondary inoculum is rain-splash, air currents and wind driven moisture (Singh *et al.*, 1997).

The use of clean seeds, field sanitation, isolation from infected reservoirs, and the use of windbreaks are suggested as cultural measures to control the disease (Allen, 1983). Foliar application of fungicides can also control the disease (Singh *et al.*, 1997).

2.1.4.1.7 Brown blotch

Brown blotch is induced by two species, namely *Colletotrichum capsici* (Syd.) Butler and Bisby and *C. truncatum* (Schw.) Andrus and Moore (Singh *et al.*, 1997). Symptoms range from seeds failing to germinate, seedling damping-off, stem or branch girdling, flowers aborting, immature pods mummifying at ends of pods and leaves showing lesions (Singh *et al.*, 1997). Primary sources of inoculum are infected seeds and infested debris and secondary sources are rain-splash, wind and air currents (Singh *et al.*, 1997). The same control as for anthracnose applies to brown blotch (Singh *et al.*, 1997). Benomyl in combination with monocrotophos are effective in reducing the brown blotch on cowpea (Olowe *et al.*, 2003). The pathogen can also be effectively controlled by application of *Trichoderma viridae* (Sacks) as a spore suspension foliar spray once or twice weekly from three days after inoculation (Bankole & Adebanjo, 1996).

2.1.4.1.8 Brown rust

Brown rust is caused by the fungus *Uromyces vignae* Barclay. Symptoms of brown rust are slightly raised brown or black pustules on the leaves (Allen, 1983; Singh & Rachie, 1985). Dispersal is through contact with people, animals, farm implements, wind and insects (Singh *et al*., 1997).

2.1.4.1.9 *Cercospora* and *Pseudocercospora* leaf spots

Cercospora leaf spot is induced by *Cercospora canescens* Ell. & Mart, while *Pseudocercospora* leaf spot is induced by *Pseudocercospora* (*Mycosphaerella*) *cruenta* (Sacc.) Deighton, formally *C. cruenta* (Emechebe & Shoyinka, 1985 as reported by Singh & Rachie, 1985). The lesions are small, brown and circular with reddish-purple borders on leaves (Boyhan *et al*., 1999). The pathogen overwinters on infected crop residues and infected seeds (Singh *et al*., 1997). Cultural practice like cop rotation and removal plant debris has been found to be effective in reducing the diseases incidence (Boyhan *et al*., 1999).

2.1.4.1.10 Powdery mildew

Cowpea is susceptible to powdery mildew during wet and humid conditions (Valenzuela & Smith, 2002). Powdery mildew is caused by *Oidium* spp., *Erysiphe polygoni* (DC) and *Sphaerotheca fuliginea* (Schelecht.) Pollacci. and it can be controlled by the use of resistant varieties and application of fungicides such as triadimefon (Singh *et al*., 1997). The symptoms are white, powdery growth consisting of oidia appearing on the upper surface of the leaf (Boyhan *et al*., 1999). Chlorotic and then brown patches also appear on the upper surface of the leaf, which finally result in the defoliation of the plant.

2.1.4.1.11 Seedling decay and damping-off complex

Seedling decay and damping-off occurs during pre- and post-emergence and they are induced by four pathogens namely: *Pythium aphanidermatum* [Edson] Fitzp, *Rhizoctonia solani*, *Fusarium solani* (Mart) App. and Wol. *Colletotrichum capsici* and *Macrophomina phaseolina* (Tassi) Goid (Dorrance *et al*., 2001). Aveling & Adandonon

(2000) also found that damping-off in South Africa is mostly caused by *Pythium ultimum* Trow and *R. solani*. *Rhizoctonia solani* symptoms are characterized by reddish-brown lesions that are usually limited to the collar regions of the hypocotyls at which point the diseased seedling collapses (Agrios, 2005). *Pythium aphanidermatum* lesions move rapidly up the hypocotyls. They appear grey-green and wet and the hypocotyls eventually collapse. *Colletotrichum capsici* infected seeds fail to germinate and seedlings collapse. The symptoms are purplish-brown lesions that girdle the stem at soil level (Singh & Rachie, 1985).

Of all the diseases caused by the pathogen *F. solani*, root rot is regarded as a serious disease in most bean production countries worldwide (Nelson *et al*., 1981). *Fusarium solani* causes seedling diseases by attacking bean seeds before germination or attacking young seedlings before or after emergence (Koening, 2002). The first symptoms on cowpea are reddish streaks on the hypocotyls and taproot, which appear a week after plant emergence (Ramusi, 2006). The reddish discolouration increases and coalesces to cover the entire belowground stem and root system, giving it a brown corky appearance. The red colour turns brown with age and longitudinal fissures develop in the cortical tissue of the affected areas. As the infection becomes severe, the entire root system may be attacked and destroyed (Davis *et al.*, 1997).

On developing plants, *F. solani* symptoms are characterized by soft dark brown or black cankers that develop on the stem nodes and may girdle the stem during disease development (Loria, 1993). Foliar symptoms develop shortly after the onset of crop flowering and they include mottling, mosaic, interveinal chlorosis and necrosis on the upper leaves, defoliation and premature plant death (Loria, 1993). The disease causes more damage to stressed plants under conditions of reduced root growth caused by drought, poor nutrition, or oxygen stress caused by wet soil (Davies *et al*., 2004). *Fusarium* is typically found on diseased seedlings, therefore seed-applied fungicides are effective in controlling *Fusarium* (Daferera *et al*., 2002).

2.1.4.1.12 Summary

Eleven major fungal diseases of cowpea have been identified among which anthracnose, ascochyta blight, damping-off, brown blotch, soft stem rot and brown rust are considered to be of greatest importance in Africa (Emechebe & Florini, 1997). In addition septorial leaf spot and scab are important constraints in humid regions. Smith *et al*. (1999) reported *C. dematium* as a cowpea disease in South Africa. Smith *et al.* (1999) further reported that the pathogen is soilborne and can be controlled through chemical seed treatment. According to Adandonon *et al.* (2004), damping-off is harmful to many cultivated crops including cowpea. Damping-off of cowpea results in yields losses with serious socio-economic implications (Adandonon *et al*., 2004).

2.1.4.2 Viral diseases

The most damaging viral diseases of cowpea are seedborne. Their symptoms generally appear most severely on the leaves, where they cause stunting and deformation (Alexandra *et al.,* 2002). Among these, several are of importance in Africa, including cowpea yellow mosaic virus, cowpea aphid-borne mosaic virus and cowpea severe mosaic virus (Cesse, 1995).

Mosaic viral diseases are the viral diseases most common in cowpea growing areas (Alexandra *et al.,* 2002). The disease symptoms are from inconspicuous light-green mottle to yellow mosaic to leaf distortion, sometimes with reduced plant growth (Cesse, 1995). According to Pouwels *et al.* (2001), cowpea mosaic virus produces chlorotic spots with diffuse borders in inoculated primary leaves. The author further mentioned that trifoliate leaves develop a bright yellow or light green mosaic of increasing severity in young leaves. The host range is rather limited, and a few hosts are known outside the Leguminosae where the virus is transmitted by various beetles (Killebrew, 2001). According to Alexandra *et al.* (2002), the yellow cowpea virus poses a great threat to the legumes. This pathogen replicates in the cowpea protoplast and systemically infects cowpea seedlings during their growth.

Most varieties bypass the damages caused by the viral disease, and succeed in producing, to a certain extent, an acceptable yield (Alexandra *et al.*, 2002). The genetics of resistance to cowpea viruses has been extensively studied, and numerous sources of resistance to cowpea viruses have been identified (Hapton *et al.*, 1997).

2.1.4.3 Bacterial diseases

Bacterial blight caused by *Xanthomonas campestris* pv. *phaseoli* and bacterial pustule *(Xanthomonas* sp.) are the two most important bacterial diseases of cowpea in Africa (Emechebe & Florini, 1997). Both pathogens are transmitted via seed, and planting infested seed often causes spread of the disease. However, good sources of resistance have been identified for both diseases (Devries & Toenniessen, 2001).

Bacterial blight is the most devastating disease of cowpea in dry regions of west and central Africa. The initial symptoms of bacterial blight are tiny water-soaked dots on leaves (Wydra & Sikiro, 1997). The dots remain small and then enlarge and became orange with a yellow halo (Singh *et al.*, 1997). When the stems are infected, the pathogen causes cracking (stem canker) and water-soaked pods from where the pathogen enters the seed and is thus seedborne (Wydra & Sikiro, 1997).

The severity of the diseases can be reduced by a mixed cultivation with cassava (*Manihot esculenta* Cruntz) or maize (Wydra & Sikiro, 1997). According to Saettler (1998), one way of minimizing the disease incidence is by using pathogen free seed. Saettler (1998) further mentioned that working in the field during wet conditions could aggravate the disease in an infected field. Good sanitation is recommended which includes collecting and burning of all infected plant debris, crop rotation with nonleguminous plants and deep plowing (Sherf, 1997). Chemical control can also be used as foliar spray using copper compounds applied weekly. Sherf (1997) further mentioned that resistant varieties could be used to minimize infection.

2.2 The pathogens

There are approximately 40 fungal species that are cowpea pathogens and nine of them have been reported in South Africa as cowpea pathogens (Van den Berg *et al*., 2001). Like other crops the economic importance of cowpea pathogens varies considerable with ecological zones and environmental conditions (Kassou *et al*., 2001). The damping-off and stem rot diseases include *R. solani, P. ultimum, F. solani Sclerotium rolfsii* Sacc., *Colletotrichum* spp and *Phytophthora* spp. that are all promoted by warm and cool temperatures (Sinha & Khare, 1977). Latuda-Dada *et al*. (1993) reported that in West Africa these diseases result in yield losses of over 60%. In Benin losses are estimated to over 44% in the northern part of the country. (Latuda-Dada *et al*. 1993). In Uganda, identification of these diseases was based on field symptoms, but little is known about the seed and soilborne pathogens (Nakawuka *et al*., 1997). However, Nakawuka *et al*. (1997) reported that such pathogens causes complete rot of seed, whereas partial infection led to pre- and post-emergence rot, root rot and hypocotyl rot. According to Food Agricultural Organisation (FAO) (1998), report losses due to diseases on legumes are estimated to over $30 billion in sub-Sahara Africa. Wrather *et al*. (1994) estimated economic losses due to pests and diseases on soybean to 14 metric tons, valued at $3.31 billion. In Nigeria, out of 3.3 millions tones of cowpea expected annually, 1.1 million tons of cowpeas, with a market value of $1.8 billion is lost as a result soilborne diseases which result in poor crop establishment (Amusa & Adegbite, 2003). Below is a discussion of the most important cowpea seedlings pathogens in southern Africa on which this study focuses.

2.2.1 *Rhizoctonia solani*

The fungus *Rhizoctonia solani* is common in most soils and attacks hundreds of different plants such as beans, cowpeas, and many more (Singh *et al*., 1997). It is subdivided into many strains and anastomosis groups that differ in the hosts and tissues they attack (Harikrishnan & Yang, 2004). *Rhizoctonia* persists indefinitely in soil as a saprophyte and survives extremes in temperature and soil moisture as small, brown, rounded sclerotia (Singh *et al*., 1997). Under favourable conditions the sclerotia germinate by

producing delicate hyphae that grow through the soil and invade roots directly, through wounds or natural openings when sufficient soil moisture is present (Singh *et al*., 1997).

Kataria & Grover (2004) reported that like all root-rotting fungi, *Rhizoctonia* is a soil inhabitant and once introduced persists in the soil for several years. It damages legumes at relatively low soil temperatures (18°C) but is most aggressive under warmer conditions (24 to 30°C). *Rhizoctonia* infection and disease development occur over a wide range of soil moistures (Singh *et al*., 1997).

The symptoms of the disease are difficult to distinguish from those caused by other seedling pathogens (Singh *et al*., 1997). Symptoms include brown to reddish brown sunken lesions on hypocotyls near or below the soil line (Ebbels, 1993). These sunken lesions, caused by the death and collapse of cortical cells, are often referred to as 'sore shin' (Agrios, 2005). The lesions may girdle hypocotyls of the host plant creating a condition referred to as "wirestem", which usually kills the seedlings (Kirkpatrick & Rothrock, 2001).

Fungicides are commonly applied as a primary seed treatment for controlling this disease (Maude, 1996). Seed treatment with carboxin was more effective than similar treatments with conventional fungicides in preventing seedling infection caused by *R. solani* on beans (*Phaseolus vulgaris* L.), pea *(Pisum sativum* L.*)* and radish (*Raphus sativus* L.) (Amin, 1991). However, Kataria & Grover (2004) found that seed treatment using the benzimidazole fungicides were more effective than carboxin or chloroneb in protecting mungbeans (*Vigna radiata* L. Wilczek) from attack by *Rhizoctonia.* This combination inhibited pathogen growth under laboratory (*in vitro*) conditions. It was also reported that pencycuron inhibited binucleated *Rhizoctonia* on wheat under greenhouse conditions (Duffy, 2000).

The inhibition of mycelial growth of *R. solani in vitro* was found to be best with pencycoron, followed by tolclofos-methyl, carbon and thiabendazole (Franeko, 2003). Tolclofos-methyl was most effective against cowpea seedling rot in soil infested with *R.*

solani followed by pencycuron, thiabenazole and carboxin. Carboxin gave better control of the pathogen when applied to cowpea seed already coated with phosphamidon or dimethoate (Franeko, 2003). A combination of pencycuron and tolclofos-methyl as a seed treatment for cowpea seeds gave nearly 100% disease control in either the presence or absence of insecticides (Kataria *et al*., 1989).

Richardson (1981) found that seed treatment of pea seed with aldrin, dieldrin and lindane together with thiram, captan or chloranil ensured better protection against *R. solani* than each fungicide alone. Leach & Franeko (1982) found that aldricarb considerably increased the level of disease control by quintozene in soil having an indigenous low inoculum level of *R. solani.* Treating cotton seed (*Gosspyium* spp.) with a combination of metalaxyl and mycobutanil reduced *Rhizoctonia* incidence and improved plant stand and emergence, regardless of soil type and population of the pathogen in the soil (Davis *et al*., 1997).

In Zimbabwe, *R. solani* in cotton was effectively controlled by seed treatment with non-systemic fungicides tolclofos-methyl and pencycuron plus captan, than by treating with non-systemic compounds carboxin and benodanil (Franeko, 2003). Azole fungicides are known to be fungistatic in action. When applied as a seed treatment it was found that they are short lived, hence quickly broken down in host tissue (Agarwal & Nema, 1994). In India, Senegal and west Africa seed treatment with benzimidazole based fungicides controlled stalk rot of bean and damping-off infection of tomato (*Lycopersicon exculentum* L.) cowpea and beans (Agarwal & Nema, 1994).

Binucleated *Rhizoctonia* cannot be inhibited by metalaxyl, but a combination of metalaxyl and binucleated *Rhizoctonia* suppressed *Phytophthora* sp. and other damping-off pathogens (Harrisson & Nelson, 1999). Abbassi *et al*. (2004) demonstrated that fish emulsion, added to pear-bases mix substrate or soil, act as disease control for damping-off diseases including those caused by *Rhizoctonia*. Fish emulsion, not only improved disease management, but was also found to be an excellent source of fertilizer.

On tobacco (*Nicotiana tabacum* L.) *R. solani* was reduced by iprodione at a rate of 1.12kgai/ha under greenhouse conditions. Gowily & Soliman (1994), in their study of *Rhizoctonia* on broad bean (*Phaseolus vulgaris* L.), found that benlate gave superior results in controlling bean damping-off and root rot caused by *R. solani*. Carbendazim was found to be more effective in controlling *R. solani* when combined with N + P + K on cowpea, but qiuntozene and methoxyethyl mercury chloride were less effective against the pathogen when fertilizer was added to the soil (Kataria *et al*., 1991).

Seed treatment with systemic fungicides such as chloroneb, which is effective against a number of damping-off organisms, has protected against root rots caused by *Fusarium* and *Rhizoctonia* spp. affecting cucumber (*Cucumis sativus* L.) and tomato in a protected cropping system (Worth & Hance, 1991).

Kataria *et al.* (1991), studying sensitivity of *Rhizoctonia* species to different fungicides, found that cyprocanazole and tolclofos-methyl were generally inhibitory to *R. solani* both *in vitro* and *in vivo*. Triadimenol and carboxin provided considerable variation in activity against different species of the pathogen. *Rhizoctonia* was also controlled by imazalil and fenarimol on soybean (*Glycine max L*. Merr). Benomyl and propiconazole showed strong activity against *Rhizoctonia* on legumes and cereals. It was also found that pencycuron had a strong inhibitory activity against *Rhizoctonia in vitro* (Kataria *et al*., 1991).

The herbicides, flochoralin and alaclor, applied to soil altered the effectiveness of fungicide treatments of methoxyethylmercury, captafol, propamocarb, quintozene and carbendazim to control cowpea damping-off caused by *R. solani* (Kataria & Dodan, 1981). Hill *et al*. (1994) also demonstrated that *Pseudomonas flourescens* Syn (strain Bl915) has an ability to inhibit *Rhizoctonia* damping-off of cotton. Chen *et al*. (1992) found that treating seed with *Streptomyces padanus* (Sacc) (strain SS07) or (strains 09 alone or combined with 1% (w/w) of soil biofungicides) significantly reduced the percentage of colonization of cabbage (*Brassica oleracea* L. var. *costana* DS) seed by *R. solani* compared to untreated controls. Combination of pencycuron and *P. flourescens*

(strain 2-79) was found to be effective against *R. solani* and take-all of wheat (*Triticum aestivum* L.) (Duffy, 2000).

2.2.2 *Pythium ultimum*

Pythium ultimum Trow belongs to the class Oomycetes (Jones & Samac, 1996). *Pythium* species cause pre- and post-emergence seedling damping-off in cowpea (Aveling & Adandonon, 2000). The genus *Pythium* is ecologically and physiologically dispersed worldwide and is found in soil, sand, pond and stream water and their sediments (Moorman, 2004). There are many known species of *Pythium*.

The symptoms of *P. ultimum* are seedling damping-off, smaller deformed primary true leaves, plant stunting, loss of fine feeder roots and poor yield (Paulitz & Adams, 2003). *Pythium* spp. are rapidly growing fungi that need minimal nutrition for growth of their hyphae (Carroll, 2004). Infected seed failing to germinate, become soft and mushy and later turn brown, shrink and finally disintegrate (Moorman, 2004). Emerged seedlings are attacked at roots or anywhere below the soil line. Infected plants are water-soaked and discoloured, and they soon collapse (Carroll, 2004). The basal part of the stem turns soft and becomes thinner than the upper parts as the fungus grows. The fungus continues to infect the fallen seedling, which withers and dies (Moorman, 2004).

The pathogen can be controlled by seed treatments. Paulitz (1991) found that treating germinating seed with *P. flourescens* controlled *Pythium* on wheat. Treating seed with a combination of both *Streptomyces* and metalaxyl inhibited the development of *Pythium* damping-off in a rolled paper towel. This also increased the frequency of healthy plants significantly for the susceptible varieties and the average severity index decreased for both resistant and susceptible varieties (Jones & Samac, 1996).

According to Harris *et al*. (1999), propamocarb reduced *Pythium* damping-off and increased shoot weight in soybeans. Under field conditions treating seed before planting with metalaxyl reduced damping-off caused by *P. ultimum* and improved seedling emergence on sweetcorn (Mathre *et al*., 1993). According to Hancock (2004), pentochoronitrobenzene (PCNB) and etridiazole was found to control *Pythium* and *Rhizoctonia* on beans when applied as an in furrow spray during planting. Wayne *et al.* (2003), studied the influence of metalaxyl fungicide seed treatments on soybean to control *Pythium* under no tillage conditions, and found that addition of metalaxyl to captan as a seed treatment significantly reduced *Pythium* compared to seed treatment with captan only. Seed treatment with metalaxyl effectively controls *Pythium* damping-off of pea (Mathre *et al*., 1993). A seed treatment with a mixture of metalaxyl and cloroneb (a systemic fungicide highly fungistatic to *R. solani*) has been used successfully to control damping-off disease complex of cotton caused by *P. ultimum* and *R. solani* (Suet, 1990).

Under greenhouse conditions metalaxyl and chlorothalonil were found to provide significant and consistent control of *Pythium* on carrots (www.syngenta.co.za a). Chlorothalonil and fosetly-Al were also found to be active against the pathogen. Under field conditions a minimum disease incidence of 5% and maximum of 95.5% germination were recorded when potato seed were treated with ridomil 0.25% for an hour to control *Pythium* (Mathre *et al*., 1993).

Treating seeds with flutolanil and metalaxyl then mixed with gel or applied as a drench, reduced the incidence of both *Pythium* and *Rhizoctonia* in mungbeans (*Phaseolus aureus* L.) under field conditions. Ghales *et al.* (1991), studying control of *Pythium* antagonistic fungal metabolites incorporated into sugar beet (*Beta vulgaris* L.) seed pellets, found that incorporating hymexazole 10.5 –14 kg/ha in seed pellets generally increased seedling establishment and slightly reduced post-emergence losses. This fungicide was also found to have no risk of phytotoxic effects on the young seedlings.

According to Ferris & Mitchel (1991), chloroneb, a systemic fungicide was found to be very effective against *Pythium* species. The study also showed that benomyl and thiram

reduced the population of *Pythium* spp. in soil and improved germination of soybean seed. According to Singh *et al*. (1997), captan when applied as seed treatment slurry, can control *Pythium* damping-off on legumes. An early season application of metalaxyl and mefenoxam on cowpea and beans can also give good control of *Pythium* damping-off (Jones & Samac, 1996). Thiram is also effective against the pathogen but it must be applied as a slurry (Singh *et al*., 1997).

2.2.3 *Fusarium solani*

Fusarium solani is a soilborne pathogen found in most soils worldwide (Nelson *et al*., 1981). It can survive as both a saprophyte and a facultative parasite, associated with wounds and other infections that cause root rot, stem cankers, and storage rots of many plants (Marasas *et al*., 1984). *Fusarium solani* causes a variety of diseases on different hosts and can attack several plant species including most greenhouse vegetables (Nishijima, 1993).

Fusarium solani is widely distributed in soil, on subterranean and aerial plant parts, and in debris (Nelson *et al*., 1981). The optimum growth temperature of *F. solani* is 27-31°C, optimum pH is 7.8 and humidity should be at 98%. (Glen *et al*., 2003). This pathogen over-winters as chlamydospores in naturally infested soil (Davis *et al*., 2004). The spores of *F. solani* are disseminated by water-splash, on pruning knives and other tools, clothing or worker's hands (Cercauskas, 2001). Humans also contribute to the dissemination of *Fusarium* pathogens through their distribution of infected or infested seeds or other plant material (Nelson *et al*., 1981).

Fusarium solani causes seedling diseases by attacking seeds prior to germination or attacking young seedlings before or after emergence. The first symptoms are reddish streaks on the hypocotyls and tap roots, which appear a week after plant emergence (Nelson *et al.,* 1981). The reddish discolouration increases and coalesces to cover the entire below ground stem and root system, giving it a brown, corky appearance. The red

colour turns brown with age and the longitudinal fissures develop in the cortical tissue of the affected areas. As the infection becomes severe, the entire root system may be attacked and destroyed (Nelson *et al*., 1981).

The pathogen can be controlled effectively using chemical seed treatments (Maude 1996). The benzimidazoles, in addition to being effective in seed treatment application against individual soilborne *Fusarium* spp., are also toxic to some fungal complexes from the soil. Goncalves *et al*. (1991a) found that seed treatment with carboxin followed by a soil drench of either fungicide effectively controlled *F. solani* and *R. solani* on cotton.

According to Koller *et al*. (1982), *F. solani* was effectively inhibited by benomyl. The results suggested that benomyl at low concentrations prevented fungal penetration and thus infection probably through inhibition of the cutinase (Koller *et al*., 1982). The authors further reported that benomyl prevented infection of soybean by conidia of *F. solani*. Methyl-2-benzimidazolecarbamates (MBC) was reported to strongly inhibit mycelial growth with 50% inhibition indicating a toxic mode of action (Goncalves *et al*., 1991b). Smiley & Craven (1994) reported that benomyl and iprodione inhibited *F. solani* on turf grass (*Agrostis palustris* Hunds).

Mancozeb has provided control for *Fusarium* spp. on seeds, tubers and seedlings of a variety of agricultural crops (Agrios, 2005). Allen (2004), studying fungicides for control of species of *Fusarium* spp. on longleaf pine (*Pinus radiata* L.) seed under greenhouse conditions, found that benomyl completely inhibited growth of *Fusarium* spp. and improved seed germination. Dithane M-45, when incorporated into agar medium, was found to inhibit the growth of *F. solani*, but under greenhouse conditions only a higher dose of 24.0 ppm was able to control the pathogen (Moubasher *et al*., 1986). Mills *et al*. (2004), in their study on the effect of salt compounds on mycelial growth, sporulation and spore germination of various potato pathogens, found that mycelial growth and spore germination of *F. solani* was strongly inhibited by sodium metabisulfite and propyl-paraben. In addition, spore germination of *F. solani* was consistently inhibited by

aluminum compounds (aluminum chloride, aluminum acetate and alum) and the commercial fungicide, mancozeb (Mills *et al*., 2004).

According to Maude (1996), seed treatment with benzimidazole-based systemic fungicides protects against infection from soil-borne *F. solani* (foot rot) and *F. oxysporum* (cotton rot). The benzimidazoles, in addition to being effective in seed treatment application against individual soilborne *Fusarium* spp., are also toxic, as are the phenylamide fungicides carboxin, to some fungal complexes from the soil. Goncalves *et al*. (1991b) found that seed treatment with carbendazim or carboxin followed by a soil drench of fungicide effectively controlle*d F. solani,* and *R. solani* on cotton.

2.3 Chemical seed treatment with fungicides

2.3.1 Introduction

Cowpea is mainly grown by small-scale farmers who store seed under poor conditions (Singh *et al*., 1997). This subjects the seed to a number of seedborne pathogens including storage pests. Kritzinger *et al.* (2002) also confirmed that storage fungi reduce germination of cowpea seed, hence causing poor crop stand which result in low yield. Treating seed with fungicides protect cowpea seed from soilborne pathogens and storage fungi (Kritzinger *et al.* 2002 ; Ramusi, 2006).

2.3.2 Seed treatment

Seed treatment is a biological, chemical, mechanical or physical process designed to mitigate externally or internally seed or soilborne microorganisms, resulting in the emergence of a healthy seedling and, subsequently healthy plant (Scot, 1989). Seeds may be treated to promote good seedling establishment, to minimize yield losses or to maintain and improve quality, and avoid further spread of the pathogen (Agarwal & Sinclair, 1996).

Seed treatment may have one or several functional objectives. Fungicide seed treatments are applied to seed to eliminate inoculum (Sinclair, 1993). This may kill or neutralize the seedborne pathogen. It also prevents transmission of the disease thus producing healthy seedlings and crops (Rennie, 1993). Fungicides and chemical seed treatments are also applied to protect germinating seeds and emerging seedlings from soilborne and airborne pathogens (Maude, 1996).

Soilborne pathogens pose a serious threat to the unprotected seed at planting (Maude, 1996). These can inhibit emergence and germination. Certain soil conditions may make the seed susceptible to the same pathogen that causes seedling diseases like seed decay, seedling blight and root rot (Ellis & Paschal, 1990). Frequently eradicative and protective functioning chemicals or fungicide seed treatments are necessary. Chemicals used in seed treatment are fungicidal when they kill fungi and fungistatic when they prevent additional growth or sporulation of an organism without killing it (Scot, 1989).

Seed treatment fungicides can be divided into two groups, non-systemic and systemic (Maude, 1996). Non-systemic fungicides are those which have limited penetrative activity when applied to the surface of the seed and generally are not mobile within the tissue of the seeds (Worth & Hance, 1991). They provide protection against invasion by soilborne fungi for a short period of time (Maude, 1996) and are mainly protectant in action but can have some eradicant effect when seeds are sown in aqueous solution or suspension of those chemicals (Wain & Carter, 1977). According to Wain & Carter (1977), systemic fungicides are defined as those compounds, which can prevent disease development on regions of the plant away from the site of application. Systemic compounds applied as seed treatments function mainly as eradicants of deep-seated infections of seeds, but they can translocate via the internal tissue of seed into hypocotyls and the developing radicle or plumule of plants and there may give added protection against external soilborne and foliar pathogens (Marshal, 1977).

2.3.3 Method of seed treatment

The methods of seed treatment include any process used for the addition of materials to seeds; in its simplest form, this is the direct application of material to seeds (Stead, 1992). Seed coating generally is used to denote the application of a material to the seed without changing its general size or shape (Taylor & Harman, 1990).

2.3.2.1 Slurry seed treatment

The active ingredient may be dispersed or suspended in water to form a slurry (Gremell & Herridge, 2004). Slurry application improves uniformity and helps overcome problems associated with dry powder application (Rennie, 1993). Slurry treatment may include the use of adhesives such as binders, glues, and stickers to improve retention of materials (Taylor & Harman, 1990). Adhesives used for application include dextran, gum, Arabic methylcellulose, paraffin or vegetable oil (Maude, 1996).

In slurry treatment, water-dispersible fungicides formulations are mixed in water so that a slurry formation results, which is applied to seed (Rennie, 1993). The slurry treatment is used primarily to treat seed after harvesting during seed processing (Maude, 1996). It is easy to differentiate between a treated and non-treated seed lot, as seed are coloured according to the chemical, for example blue or red (Rennie, 1993). Most fungicides used for seed treatment can be treated using the slurry method (Nene & Thapliyal, 1993). Fungicides can also be mixed with other biological agents e.g. *Pythium* spp. *Rhizoctonia* root rot can be effectively controlled using a slurry treatment of captan combined with the fungus *Trichoderma harzianum* Rafai. (Ruppel & Baker, 1998).

2.4 The fungicides

2.4.1 Thiram

Thiram is a seed protectant fungicide registered for use on all important field crops (Bhardwaj & Shrestha, 1985). It is an organic sulphur compound, grouped under

dithiocarbamates. This is one of the most important, versatile and widely used groups of modern fungicides (Agrios, 2005). These derivatives of dithiocarbamates are toxic to fungi because they are metabolites of the isothiocyates radical. This radical inactivates the sulfhlydryl groups (-SH) in amino acids and in enzymes within pathogen cells thereby inhibiting the production of these compounds (Agrios, 2005).

Plants treated with thiram, or any mixture containing thiram, remain free from seed diseases and have a higher grain yield than untreated seed (Anaso *et al.*, 1988). Kataria & Grover (1991) found that treating soybean seed with thiram did not have any phytotoxic effect. Seed treatment with thiram was reported to increase pea stands compared to the non-treated control (Pendulosi, 1997). Franeki & Bratuli (1994) reported that thiram reduces rust and chocolate spot on beans. A seed treatment of broad beans with thiram and endosulphan reduced the severity of seedborne diseases and leaf fly (Miller, 1994). Furthermore they reported that a combined treatment increased yield by 17%. The study also found that thiram was effective over a broader range of soil conditions. Thiram application as a seed treatment on cotton seed controls many seedborne pathogens (Montermayor, 1995). Its application on soybean was found to effectively control stem gall and it increased yield of the crop (Bhardwaj & Shrestha, 1985).

In Israel combining thiram and pencycuron as a seed treatment before planting effectively reduced *R. solani,* which frequently induces seedling diseases in cotton fields. Captan and thiram was also reported to improve emergence (Whitehead, 1995). In Iraq *R. solani* was controlled by combining vitavax with thiram as a seed treatment on hemp (*Cannabis sativa* L.) (Montermayor, 1995). Song *et al*. (2003) found thiram and prochloraz were effective in inhibiting mycelial growth of some *Fusarium* species when studying tomato wilt and its chemical control strategies in hydroponics. Pendulosi (1997) reported that *F. solani* and *R. solani* were effectively controlled by thiram under laboratory conditions.

Payne & Williams (1990) reported that seed establishment and post emergence losses caused by *Pythium* species on sugar beet could be improved by seed treatment with thiram and hymexazole. Steeping seed in 0.2% suspension of thiram improved yield of

sugar beet when thiram was used to replace diethyl mercuric phosphate for controlling diseases of sugar beet (Durant *et al*., 1988).

2.4.2 Celest® XL

2.4.2.1 Fludioxonil

The product contains a broad-spectrum seed treatment fungicide fludioxonil as an active ingredient and contains mefenoxam (www.syngenta.co.za a). Fludioxonil has a wide registration on major food crops such as legumes, corn, potato, rice (*Orizae sativa*), vegetables and cereals (Zang *et al*., 2001). Fludioxonil belongs to the phenylpyrrole class of chemicals and has a unique mode of action (Zang *et al*., 2001). It is the most commonly used fungicide in Europe as a seed protectant on cereals and legumes. It gives an excellent start to the crop and germination is secured (Cahill, 2000). Furthermore it is safe to humans and the environment. It is also fully compatible to use with other fungicides e.g. Cruiser, Gaucho, Apron and other highly valued seed treatment products (Cahill, 2000)

Fludioxonil can effectively be used to control *Pythium* spp. and *R. solani* on seedlings of apple (*Malus domestica* Borkh) (Mazzola, 1998). Grey mould and brown rot was also controlled in cherries (*Prunus avium* L.) by using fludioxinil and tebuconazole (Stensvand & Borve, 1999). Butcher & Pedersen (2004) found fludioxonil to be effective against *R. solani* on soybean. McGovern *et al*. (2001) reported that mefenoxam is effective in reducing disease caused by *Fusarium* spp. Zang *et al*. (2001), indicated that fludioxonil has demonstrated activity against several species of *Fusarium* that causes damping-off in maize.

Inglis *et al*. (1999) reported that fludioxonil and mancozeb could control Late blight (*Phytophthora infestans* Dec.) in potatoes when applied as protective fungicides under greenhouse conditions. Stensvand (1998) reported that fludioxonil is an optimum choice for intensive cereal growing. It offers best available control of *Fusarium* spp. causing a major seedling disease of cereals in Western and Central Europe, as well as unrivalled

control of other seedling diseases such as bunt of wheat and septorial leaf spot (Cahill, 2000).

2.4.2.2 Metalaxyl (mefenoxam)

Metalaxyl is a low-rate phenylamide systemic fungicide registered for the control of damping-off and seed rot diseases and is considered to be highly effective when used as a seed treatment against fungi belonging to the class Oomycetes (www.syngenta.co.za b). Metalaxyl is used to provide full protection to the seed and seedlings during the growing period (www.syngenta.co.za b). The fungicide has previously been found to be effective against most of the diseases caused by *Phytophthora* and *Pythium* spp. as reported by Farith *et al*. (1981), Whang & Kim (1995), Peters *et al*. (2001) and Malvick & Grunden (2004). Bhaskar *et al*. (2005) reported similar results for the ability of metalaxyl to reduce the diseases caused by *F. solani*. Mixtures of metalaxyl and fludioxonil are effective in reducing diseases caused by *F. solani* (Wang *et al*., 2005). Fisher & Hayes (1982) and Brantner & Windels (1998) also reported metalaxyl to be effective in reducing diseases caused by *P. ultimum*. Harris & Nelson (1999) also conducted an *in vitro* experiment and found that metalaxyl was capable of inhibiting the mycelial growth of *R. solani*.

2.5 Conclusion

Cowpea is susceptible to a wide range of pest and pathogens, which can cause damage to the crop at all stages of growth (Summerfield & Roberts, 1985). Seedling diseases in cowpea results in low yields, especially in rural areas where no control measures are taken against these pathogens. Chemical seed treatment can play an important role in seed pathology and general disease control. Fungal diseases such as like *R. solani, P. ultimum* and *F. solani.* are a major problem associated with cowpea production in southern Africa (Cesse, 1995). Treating cowpea seeds to control seed and seedling diseases prior to planting is one method that has not been explored in southern Africa. As a result there is presently no fungicide registered to control seed and seedling diseases on cowpea in southern Africa.

There is a need therefore, to evaluate Celest® XL and thiram as seed treatment fungicides to control *R. solani, F. solani* and *P. ultimum* on cowpea. This study can aid with the registration of these fungicides as seed treatments on cowpea.

CHAPTER 3

EFFICACY OF THIRAM AND CELEST® XL AGAINST *RHIZOCTONIA SOLANI*, *FUSARIUM SOLANI* AND *PYTHIUM ULTIMUM IN VITRO*

3.1 Introduction

Rhizoctonia solani (Kuhn), *Pythium ultimum* (Trow) and *Fusarium solani* (Mart) App. and Wol. are important pathogens of cowpea (*Vigna unguiculata* (L.) Walp). These pathogens contribute to a significant yield reduction in the crop (Adandonon *et al*., 2001). Seed treatment is one of the methods increasingly being used to control diseases in many crops including legumes (Van den Berg *et al*., 2001). Presently there is no fungicide registered as a seed treatment for cowpea in South Africa. In a study evaluating different fungicides to control *Colletotrichum dematium* (Pers), Smith *et al.* (1999) indicated that fludioxonil and thiram were effective in inhibiting mycelial growth of this pathogen of cowpea.

Xue (2003) indicated that thiram was effective in reducing mycelial growth of *P. ultimum* and *F. solani*. Similar findings were reported by Fravel *et al*. (2004) who reported thiram to be effective *in vitro* against *Fusarium* species. The ability of thiram to control seedborne pathogens has resulted in the registering of this fungicide on beans (*Phaseolus vulgaris* L.) in South Africa (www.syngenta.com a).

Celest® XL has shown promising results to control seedborne diseases when used as a seed treatment (Mathre *et al*., 1993). From past studies it is evident that Celest® XL has an ability to control seedborne diseases such as *P. ultimum*, *R. solani* and *F. solani* (www.syngenta.com b). The composition of this fungicide allows the control of *P. ultimum*, because it contains mefenoxam (metalaxyl) as well as fludioxonil as active ingredients, which are also known to be effective against both *F. solani* and *R. solani* (www.syngenta.com b). Findings like those of Wang *et al.* (2005) indicate that a mixture

of fludioxonil and metalaxyl completely inhibited *F. solani* while Jones (2000) found fludioxonil and mancozeb to inhibit *Fusarium* species. The effectivity of this fungicide makes it suitable for a trial on cowpea pathogens.

The idea of introducing Celest® XL as a seed treatment fungicide for cowpea focuses at minimizing losses incurred by small-scale farmers due to *P. ultimum, R. solani* and *F. solani*. Mauricio *et al.* (2005) found that mycelial growth of *R. solani* was inhibited by fludioxonil and strobilurines *in vitro.* Similar findings were reported by Kataria *et al.* (2002) who indicated that combining fludioxonil with tebuconazol completely inhibited *R. solani* under laboratory conditions.

This study was undertaken to compliment the little research that has been done in South Africa in evaluating seed treatments for the control of seedborne diseases of cowpea. The efficacy of Celest® XL and thiram to control the three pathogens, *R. solani, F. solani* and *P. ultimum, in vitro* under laboratory conditions was evaluated.

3.2 Materials and methods

3.2.1 *In vitro* study

Two pathogens *Rhizoctonia solani* (UPGH122), and *Fusarium solani* (UPGH112), were obtained from the fungal collection in the Department of Microbiology and Plant Pathology at the University of Pretoria, Pretoria, South Africa. *Pythium ultimum* (UPGH050) was obtained from a commercial hydroponics system in Pretoria. Fungicides Celest® XL [fludioxonil (25gai/L), mefenoxam (10gai/L)] and Thiram (thiram 500gai/kg DS) were supplied by Syngenta South Africa (Pty) Ltd.

Potato dextrose agar (PDA) was augmented at 1x (0.06ml), 1.25x (0.75ml) and 2x (0.12ml) the recommended rate Celest® XL/L medium and thiram at the recommended rate of 0.27mg/L medium. The media were then poured into Petri dishes (90mm) and

allowed to solidify. A mycelial disc (5mm diameter) of a seven-day-old culture of each of the three fungi was used for sub-culturing. The mycelial disc was placed in the centre of the Petri dish. The media was not amended in the control. There were five replicates per treatment. The plates were sealed with parafilm and incubated under fluorescent light at 25ºC for a total of nine days. Colony diameter was measured in millimeters across two diameters on the 3rd, 6th, and 9th day of incubation. The experiment was repeated.

3.2.2 Statistical analysis

Data was analysed using the analysis of variance test, and significant differences were determined using the Student t-test ($P \leq 0.05$).

3.3 Results

3.3.1 *Pythium ultimum*

The results of the efficacy of the two fungicides are shown in Table 3.1. All treatments were able to inhibit mycelial growth of *P. ultimum* to levels significantly less than the control (26.8mm). Celest® XL at 2x the recommended rate gave the best results (0.0mm) as it completely inhibited growth throughout this study. Thiram was the second most effective. Growth on plates augmented with Celest® XL at 1x (7.4mm) and 1.25x (7.6mm) the recommended rate did not differ from each other on the 3rd day but were significantly lower than the control. On the 6th and 9th day Celest® XL 1.25x (18.0mm and 19.4mm respectively) was more effective inhibiting mycelial growth of *P.ultimum* (Table 3.1).

3.3.2 *Fusarium solani*

All the treatments effectively inhibited mycelial growth of *F. solani* (4.4, 9.8, 8.2 and 1.6mm) and growth measured was significantly less than the control (25.8mm) after three

days. Growth on plates augmented with Celest® XL at 2x (1.6mm) the recommended rate inhibited mycelial growth the most, followed by thiram (Table 3.1).

3.3.3 *Rhizoctonia solani*

All the treatments effectively inhibited mycelial growth of *R. solani* on the 3rd, 6th and 9th day. The growth measured for all plates was significantly less than the control (90mm). Thiram (7.5mm) and Celest® XL at 2x (6.4mm) the recommended rate gave the best results and did not differ significantly from each other throughout the study. Celest® XL at 1x (26.2mm) and 1.25x (19.0mm) the recommended rate did not differ from each other on the 3rd day but growth measured was significantly lower than the control. On day 6 and 9 Celest® XL at 1.25x (18.0 and 19.0mm) the recommended rate was more effective than Celest® XL at 1x (25.2 and 26.2mm) the recommended rate (Table 3.1).

Table 3.1 Colony diameters of *Pythium ultimum*, *Fusarium solani* and *Rhizoctonia solani* grown on potato dextrose agar amended with thiram and different concentrations of Celest® XL

		Colony Diameter (mm)		
Experimental Days		3rd Day	6th Day	9th Day
Pythium				
Control		26.8d**	60.2e	90e
Thiram		3.8b	5.2b	5.4b
*Celest® XL	1x	7.4c	23.4d	24.4d
	1.25x	7.6c	18.0c	19.4c
	2x	0.0a	0.0a	0.0a
LSD		1.606	1.224	1.990
Fusarium				
Control		25.8d	61.5d	90e
Thiram		4.4b	6.8a	7.5b
*Celest® XL	1x	9.8c	25.6c	27.6d
	1.25x	8.2c	19.2b	19.6c
	2x	1.6a	5.4a	5.6a
LSD		1.973	1.674	1.770
Rhizoctonia				
Control		25.8c	58.9d	90d
Thiram		1.6a	5.6a	6.4a
*Celest® XL	1x	3.8b	25.2c	26.2c
	1.25x	4.8b	18.8b	19.0b
	2x	1.6a	5.6a	6.4a
LSD		1.334	1.224	1.428

*Celest® XL 1x = 0.06ml/L Celest® XL 1.25x = 0.075ml/L Celest® XL 2x = 0.12ml/L
**Values within a column not followed by the same letter are significantly different from each other ($P \leq 0.05$) according to the Student's t-test.

3.4 Discussion

Celest® XL was effective in inhibiting mycelial growth of *P. ultimum, R. solani* and *F. solani* in the current study. The high fungal inhibition by Celest® XL was due to the 25% mefenoxam which it contains and is a registered fungicide to control *Pythium* (www.syngenta.com b). According to McGovern (2001), mefenoxam is a broad-spectrum fungicide and Tsror *et al.* (2005) found mefenoxam to be effective in inhibiting mycelial growth of *P. ultimum* on soybean (*Glycine max* L. Merr). The results of this study also concur with those of Chatterton *et al.* (2003) who reported that metalaxyl and captafol were effective in inhibiting the growth of *P. ultimum* in an *in vitro* study on beans (*Phaseolus vulgaris* L.). Brentner & Windel (1998) found similar results when using metalaxyl on *P. ultimum*.

Fludioxonil, which is the other active ingredient in Celest® XL, is known to be effective against *Fusarium* and *Rhizoctonia* species (www.syngenta.com b). Similar findings were indicated in this study where Celest® XL was able to inhibit mycelial growth of *P. ultimum, F. solani* and *R. solani*. The results of this study also agree with Wang *et al.* (2005) who indicated that a mixture of fludioxonil and metalaxyl completely inhibited *F. solani*. Jones (2000) also shared the same sentiments and reported that fludioxonil and mancozeb inhibit *Fusarium* species. The findings of this study were also similar with that of Read & Hide (1995) who indicated that mefenoxam is effective in reducing disease caused by *Fusarium* spp.

Celest® XL at 1x and 1.25x the recommended rate were less effective in inhibiting mycelial growth of *P. ultimum, F. solani* and *R. solani* due to the lower concentrations. When the concentration of Celest® XL was doubled (2x), there was a significant decrease in the growth diameter of *P. ultimum, F. solani* and *R. solani*. Similar results were indicated by Smith *et al.* (1999).

Thiram a standard fungicide registered on bean (www.syngenta.com a) also gave good results in controlling *P. ultimum, F. solani* and *R. solani*. The results in this study concur

with results of Ebbels (1993) who found that artificial inoculation of soil with thiram protected peas (*Pisum sativum* L.) from *P. ultimum,* which causes pre-emergence damping-off and *R. solani,* which causes damping-off. Similar findings were reported by Koch (1997) who indicated that thiram reduces growth of *P. ultimum, in vitro,* when evenly mixed in potting substrates. Song *et al.* (2003) also found thiram and prochloraz to be effective in inhibiting mycelial growth of some *Fusarium* species. Similar findings were reported by Xue (2003) where the author reported that *F. solani* was greatly inhibited by thiram. The ability of thiram to inhibit mycelial growth of *P. ultimum, F. solani* and *R. solani* can be attributed to the fact that it is a derivative of the dithiocarbamates, which are toxic to fungi because they are metabolites of the isothiocyates radical (Agrios, 2005).

This study shows that thiram and Celest® XL can effectively inhibit growth of *P. ultimum, F. solani* and *R. solani in vitro*. As the concentration of Celest® XL increased there was also an increase in its ability to inhibit mycelial growth of the three pathogens, especially *R. solani.* These results clearly prove that there is a potential use for Celest® XL as a seed treatment of cowpea. However, before Celest® XL can be recommended as a seed treatment, its effect on germination of cowpea seeds need to be evaluated. This is addressed in the next chapter.

CHAPTER 4

EFFECT OF THIRAM AND CELEST® XL ON GERMINATION OF COWPEA SEEDS

4.1 Introduction

Soilborne pathogens like *Pythium ultimum* (Trow), *Fusarium solani* (Mart) App. and Wol. and *Rhizoctonia solani* (Kuhn) pose a serious threat to the unprotected seed at planting (Maude, 1996). Seed treatment is one method that can be used to minimize damage caused by these pathogens (Mankvold & O'Mara, 2002). Seed treatments are fungicidal when they kill fungi and fungistatic when they prevent additional growth of an organism without killing it (Rennie, 1993). Seedlings may be treated to promote good seedling establishment, to minimise yield losses or maintain and improve quality, and avoid further spread of the pathogen (Agarwal & Sinclair, 1996). One of the most common problems encountered in seed treatments is the effect of the various fungicides on germination (Bierman *et al.*, 2006). Certain fungicides are known to improve germination of cowpeas while others are known to be phytotoxic to the seed and seedling. Effective seed treatments must eliminate the pathogen without being toxic to the seeds (Van den Berg *et al.*, 2001).

The ability of Celest® XL to control mycelial growth of *P. ultimum*, *F. solani* and *R. solani* has to be further investigated regarding its effect on germination, shoot and root length and also percentage abnormal and diseased seedlings. It has been reported in the literature that mefanoxam (metalaxyl) and fludioxonil, which are the active ingredients in Celest® XL, do not have phytotoxic effects on germination (www.syngenta.com b). Cahill (2000) indicated that treating seeds with fludioxonil gives an excellent start to the crop germination as germination is secured, furthermore, it is safe to humans and the environment (Cahill, 2000). Similar observations were made by Mauricio *et al.* (2005) who indicated that treating seed with fludioxonil, a preventative fungicide, improved seedling emergence and increased root and shoot length of soybean (*Glycine max L.*).

Thiram and fludioxonil reduced percentage diseased and abnormal seedlings of cowpea (Smith *et al*. 1999). Read & Hide (1995) indicated that metalaxyl improved germination of legumes.

Cowpea seeds are susceptible to fungal contamination especially when stored under poor conditions together with high relative humidities and high temperatures (Kritzinger *et al*., 2002). Most small-scale farmers select their seed from the previous season's crop, which is highly susceptible to poor storage. It is well known that storage fungi are one of several causes leading to decrease in germination of seed (Singh *et al*., 1997).

The objective of this study was to evaluate the effect of the fungicides Celest® XL and thiram on seed germination, root and shoot length, abnormal and diseased seedlings.

4.2 Materials and methods

4.2.1 Germination test

The germination test was performed according to the rules of the International Seed Testing Association (ISTA)(2005). Cowpea seeds (cv. Pietersburg Blue) were obtained from Dry Bean Seed Producer's Organization, Pretoria, South Africa. This is the most common cultivar used by most farmers in South Africa. All fungicides were supplied by Syngenta South Africa (Pty) Ltd. Thiram DS (thiram, 500gai/L) was applied as a seed slurry treatment at a concentration of 0.6g/500g seed. Three concentrations of Celest® XL [fludioxonil (25gai/L) and mefenoxam (10gai/L)] were used namely, 1x recommended rate (100ml/100kg seed), 1.25x the recommended rate (125ml/100kg seed) and 2x the recommended rate (200ml/100kg of seed). The slurry was prepared by dissolving the active ingredient in water. Only water was used to wet the fungicides, no adhesive or stickers were added. Seed was soaked in water for 1min and then the required volume of Celest® XL was added and the seeds were mixed for 3-5min. The control was treated with water using the same procedure. All treated and control seeds were dried on a

laminar flow bench. Four replicates of 25 seeds were placed onto three layers of germination paper (25cm x 80cm) (Agricol South Africa (Pty) Ltd). The paper was wet in a tray containing tap water, the uppermost layer was removed and 25 seeds were placed equidistant apart on top of the other two layers, the uppermost layer was replaced and the paper was rolled. All replicates were sealed in a plastic bag and placed upright in a box at 20^{o}C for germination to occur. Each treatment was replicated four times and all treatments were treated at the same time. The experiment was repeated twice. After eight days the following data was collected according to ITSA rules (ISTA, 2005); percentage germination, shoot and root length in millimeters, and percentage abnormal and diseased seedlings. Data was statistically analyzed as described in Chapter 3.

4.3 Results

The results of the effect of thiram and Celest® XL on the germination of cowpea seed are shown in Table 4.1. All the treatments had a significantly higher germination than the control. Celest® XL 2x the recommended rate had the highest germination percentage (79%) followed by thiram (78%). Similar results were observed with regard to shoot length. Shoot lengths of all treatments were significantly higher than the control. Again Celest® XL at 2x the recommended rate had the longest shoot length (181mm) followed by thiram (174mm). Root length was significantly increased by all treatments compared to the control. Seedlings treated with Celest® XL 2x the recommended rate had the longest root length (75mm). In contrast, of the treated seeds, seedlings treated with thiram had the shortest (65.9mm). All the treatments had a significantly lower number of abnormal seedlings when compared to the control (27%). A similar trend was observed with percentage diseased seedlings. Disease incidence was significantly lowered by all the treatments when compared to the control. Thiram was the least effective in reducing disease incidence of cowpea seeds (Table 4.1).

Table 4.1 Effect of thiram and different concentrations of Celest® XL on seed germination percentage, shoot and root length and percentage abnormal and diseased seedlings of cowpea

Treatment		Germination (%)	Shoot Length (mm)	Root Length (mm)	Abnormal (%)	Diseased (%)
Control		64a**	130a	55a	27c	13.6b
Thiram		78c	174cd	65.9b	16a	3.5a
*Celest® XL	1x	68b	156.5b	71bc	19.9b	2.9a
	1.25x	68b	157b	71bc	18.5b	2.6a
	2x	79c	181d	75cd	19.8b	2.3a
LSD		3.49	4.9	5.1	2.14	2.1

*Celest® XL 1x =100ml/100kg seed, Celest® XL 1.25x =125ml/100kg seed, Celest® XL 2x = 200ml/100kg seed

**Each value is a mean of 4 replicates of 25 seeds. Values within a column not followed by the same letter are significantly different from each other ($P \leq 0.05$) according to the Student's t-test.

4.4 Discussion

Treating seed with Celest® XL and thiram resulted in an improved germination percentage. Improved germination may have been due to the fungicide seed treatment with thiram and Celest® XL, which reduces storage fungi (Alabi *et al*., 1986; Alabi & Emechebe 1990; Smith *et al*., 1999). Celest® XL contains fludioxonil, which belongs to the phenylpyrrole class of chemicals and has a unique mode of action (Cahill, 2000). The results of this study concur with Cahill (2000) who indicated that fludioxonil gives an excellent start to the crop and germination is secured. Mankvold & O'Mara (2002) indicated that treating maize seed with fludioxonil and captan improved germination. The finding of this study also concur with those of Jones & Samac (1996) who indicated that treating seed with a combination of both *Streptomyces* and metalaxyl inhibited the development of *Pythium* damping-off in a rolled paper towel and increased the frequency of healthy plants significantly for susceptible varieties.

There was no phytotoxicity expressed in the present study even at the 2x the recommended rate. Van den Berg *et al*. (2001) reported that an effective seed treatment must be able to eliminate the pathogen without being toxic to seed. Similarly, Kataria *et al.* (2002) indicated that treating soybean (*Glycine max* L. Merr) and cucumber (*Cucumis sativus* L.) seedlings with fludioxonil and tebuconazole enhanced seedling emergence. These fungicides also reduced disease severity on beans by 90% (Kataria *et al*., 2002). Wang *et al*. (2005) echoed the same sentiments and indicated that fludioxonil and *Trichoderma* could be integrated to reduce disease incidence and improve seedling establishment in the greenhouse.

The improved germination of thiram treated seeds and increased shoot length found in the present study concur with the findings of Xue (2003) who reported that thiram increased germination and shoot length of peas (*Pisum sativum* L.) by 33% and 29%, respectively. The author further revealed that the fungicide was able to reduce root rot severity caused by *P. ultimum* on peas by 65%. Furthermore, Whitehead (1995) indicated that captan and thiram improved emergence of soybean and Montermayor (1995) found that vitavax with thiram as a seed treatment on hemp (*Cannabis sativa* L.) controlled *R. solani* and resulted in improved crop establishment. Bierman *et al.* (2006) also reported that treating seed with thiram and captan improved seedling establishment. Similar results were observed in this study when cowpea seeds were treated with thiram.

The low germination of the control in the current study may have been due to storage fungi and seedborne pathogens (ISTA, 2005). This finding agrees with Kritzinger *et al.* (2002) who indicated that storage fungi reduced cowpea seed germination. Storage fungi and seedborne pathogens also caused seedlings to have shorter root and shoot length, and a high percentage of abnormal and diseased seedlings (Kritzinger *et al.,* 2002). The improved shoot and root length is probably due to the Celest® XL and thiram treatments, which contain broad-spectrum fungicides. This agrees with the study conducted by Smith *et al.* (1999) where the researchers found that thiram and fludioxonil improved shoot and root length in cowpeas.

This study conducted on germination showed that treating cowpea seed with both thiram and Celest® XL did not have any phytotoxic effect on germination. Seed treatments with thiram and Celest® XL also increased the shoot and root length of cowpea. Since the fungicides were found not to be toxic to the seedling even at double the recommended rate, these treatments can be evaluated in the greenhouse before being considered for registration on cowpea. This is addressed in the next chapter.

CHAPTER 5

EFFICACY OF THIRAM AND CELEST® XL IN CONTROLLING *RHIZOCTONIA SOLANI*, *FUSARIUM SOLANI* AND *PYTHIUM ULTIMUM* UNDER GREENHOUSE CONDITIONS

5.1 Introduction

Cowpea (*Vigna unguiculata* [L.] Walp.) is the most economically important traditional legume crop in Africa (Zohri & Gaward, 1992). Various studies in southern Africa have emphasized the need to develop commercial crops from indigenous plants, which are well suited for cultivation in areas of low agricultural potential, due to low unreliable rainfall (Aveling *et al*., 2001). However, there are several reports of seedborne fungi associated with this crop (Van den Berg *et al.,* 2001). Great losses of cowpea yield occur in the low altitude rainforest because of seedling decay and seedling damping-off (Singh & Allen, 1997). Cowpeas are susceptible to fungal diseases caused by pathogens such as *Rhizoctonia solani (*Kuhn), *Pythium ultimum* (Trow) and *Fusarium solani* (Mart) App. and Wol (Ramusi, 2006). These seedling diseases can cause damage to the crop resulting in total loss of yields (Adandonon *et al*., 2003).

Seed treatment is one method that is increasingly being used in controlling some seedborne diseases and it has shown great potential (Rennie, 1993). Presently thiram is the only registered fungicide as a seed treatment on beans (www.syngenta.com a). Thiram, which is used as a standard fungicide in this study, has proven efficacy on several legumes (Singh & Allen, 1997). According to Miller (1994), a seed treatment of broad beans (*Phaseolus vulgaris* L.) with thiram and endosulphan reduced the severity of seedborne diseases and leaf fly. Anaso *et al.* (1988) indicated that seed treated with thiram or any mixture containing thiram remain free from seed diseases and has a higher grain yield than untreated seed. Similar observations were made by Kataria & Grover (1991) who found that treating soybean (*Glycine max* L. Merr) seed with thiram did not have any phytotoxic effects. Seed treatment with thiram has also been reported to

increase pea (*Pisum sativum* L.) stands compared to a non-treated control (Pendulosi, 1997).

Celest® XL is a new fungicide that contains fludioxonil and mefenoxam (metalaxyl). Fludioxonil is effective against *R. solani* and *F. solani*, while mefenoxam controls *P. ultimum* (www.syngenta.com b). Butcher & Pedersen (2004) found fludioxonil to be effective against *R. solani* on soybean, while Zang *et al.* (2001) indicated that fludioxonil has demonstrated activity against several species of *Fusarium* that cause damping-off in maize (*Zea mays* L.). McGovern *et al*. (2001) reported that mefenoxam was effective in reducing disease caused by *P. ultimum* and *Fusarium* spp. Smith *et al.* (1999) and Van den Berg *et al*. (2001) both used fludioxonil as a fungicide seed treatment with positive results to control the two cowpea diseases.

The objective of this study was to investigate the efficacy of thiram and Celest® XL as seed treatment fungicides in controlling *R. solani*, *F. solani* and *P. ultimum* on cowpea in greenhouse trials.

5.2 Materials and Methods

5.2.1 Seed treatment

Cowpea seeds (cv. Pietersburg Blue) were obtained from Dry Bean Seed Producer's Organisation, Pretoria, South Africa. Both fungicides were supplied by Syngenta South Africa (Pty) Ltd. Thiram DS (thiram, 500gai/L) was applied as a seed slurry treatment at a concentration of 0.6g/500g seed. Three concentrations of Celest® XL [fludioxonil (25gai/L) and mefenoxam (10gai/L)] were used namely, 1x the recommended rate (100ml/100kg seed), 1.25x the recommended rate (125ml/100kg seed) and 2x the recommended rate (200ml/100kg of seed). The slurry was prepared by dissolving the active ingredient in water. Only water was used to wet the fungicides, no adhesive or stickers were added. Seeds were soaked in water for 1min at which the pre-described volume of Celest® XL was added and mixed for 3-5min. The control was treated with water using the above procedure. Seeds were allowed to dry on a laminar flow bench.

5.2.2 Pathogens

Cultures of *Rhizoctonia solani* (UPGH122) and *Fusarium solani* (UPGH112) were obtained from the culture collection of the Department of Microbiology and Plant Pathology, University of Pretoria, Pretoria, South Africa. *Pythium ultimum* (UPGH050) was obtained from a commercial hydroponics system in Pretoria. Cultures were maintained on potato dextrose agar (PDA) at 20^{o}C. Discs (5mm diameter) cut from the active growing region of seven day-old *P. ultimum R. solani* and *F. solani* cultures, using a cork borer were used as inoculum.

5.2.3 Infection studies

Pasteurised growing medium (Braaks, Pretoria) was used to fill 128-cell polystyrene seedling trays (67cm x 34cm x 8.5cm). The growing medium was drenched with tap water a day before pathogen inoculation. A single cowpea seed along with two mycelial discs were planted at a depth of 1.5cm in each cell. Appropriate controls in infested growing medium were also set up. The negative control was inoculated and soil planted with untreated seeds. The positive control was uninoculated soil planted with untreated seeds. There were four replicates of 25 seeds. Seedling trays were placed in a complete randomized block design in a controlled environment room at 22-25^{o}C with a natural dark/light regime. The trays were watered daily to maintain field capacity.

5.3 Data collection

Percentage emergence was counted per replicate per treatment and the average calculated. Shoot lengths were measured manually, from the soil level, a day before harvesting and the averages calculated. The seedlings were harvested on the 35th day after planting. Entire seedlings were removed and the growing media was washed off the roots in clean water to detect the disease infection on seedlings and their roots. The number of diseased seedlings was recorded. Re-isolation was not done in this study because the experiment was carried out on sterilised soil and trays were disinfected with jik before planting and diseased seedlings were compared with the uninoculated control. Roots were

then excised from the shoots with a pair of scissors and placed into labeled brown paper bags (28 x 15cm). The shoots and roots were then dried for 48hr in a fixed featured drying oven (custom made) at 65^{o}C at the Department of Botany, University of Pretoria. The dry weight of roots and shoots were recorded. Data was statistically analysed as previously described in Chapters 3.

5.4 Results

5.4.1 *Pythium ultimum*

The results of percentage emergence and diseased plants, plant height, and root and shoot dry mass is given in Table 5.1. All treatments significantly increased percentage emergence when compared to the inoculated control Furthermore, they were all not significantly different from the uninoculated control. Percentage disease incidence was significantly reduced by all the treatments, but all treatments were significantly higher compared to the uninoculated control. Plant height and dry shoot and root mass were significantly increased by all the treatments when compared to the inoculated control and they were not significantly different from the uninoculated control, except for Celest® XL treated seed which as significantly different from the uninoculated control with regard to root mass.

5.4.1.1 Symptoms

At harvesting it was observed that some seed failed to germinate, they were brown and water-soaked, whereas some seedlings showed symptoms of root rot and stunting caused by *P. ultimum*. The basal part of the stem was soft and reduced in diameter when compared to the upper part of the stem (Figure 5.1).

Figure 5.1 Disease symptoms caused by *Pythium ultimum* on cowpea seedlings (a); and non-infected cowpea seedlings (b).

Table 5.1 Percentage emergence and diseased plants, plant height, and root and shoot dry mass of cowpea seed treated with thiram and Celest® XL and inoculated with *Pythium ultimum*

Treatments	Emergence (%)	Diseased plant (%)	Height (cm)	Dry shoot mass (g)	Dry root mass (g)
Uninoculated Control	91.0b**	2.0a	10.8b	10.6b	2.4d
Inoculated Control	18.0a	51.3c	5.8a	6.75a	0.74a
Thiram	78.0b	13.0b	9.5b	11.6b	2.1cd
*Celest® XL 1x	76.0b	17.8b	9.0b	10.9b	1.4b
1.25x	80.5b	15.8b	10.0b	11.7b	1.7bc
2x	84.25.b	12.5b	10.3b	13.7b	1.4bc
LSD	15.80	5.80	3.20	3.85	1.08

*Celest® XL lx =100ml/100kg seed, Celest® XL 1.25x =125ml/100kg seed and Celest® XL 2x = 200ml/100kg seed
**Each value is a mean of 4 replicates of 25 seeds. Values within a column not followed by the same letter are significantly different from each other ($P \leq 0.05$) according to the Student's t-test.

5.4.2 *Rhizoctonia solani*

The results of percentage emergence and diseased plants, plant height, and root and shoot dry mass is given in Table 5.2. Percentage emergence was significantly increased by all treatments when compared to the inoculated control, but all treatments were significantly lower than the uninoculated control (Table 5.2). The number of diseased plants was significantly reduced by all treatments when compared to the inoculated control, but none of the treatments were significantly lower than the uninoculated control, they were all significantly higher. Only Celest® XL at 2x and 1.25x the recommended rate were able to significantly increase plant height above that of the inoculated control. The plant height of the uninoculated control differed significantly from the plants that were treated. Dry shoot mass was not significantly increased by the treatments, when compared to the inoculated control. Dry mass of roots was significantly increased by all treatments except Celest® XL at 1x the recommended rate when compared to the inoculated control. Both shoot and root mass were significantly lower when compared to the uninoculated control.

5.4.2.1 Symptoms

During harvesting it was observed that seeds that did not germinate were brown, and water-soaked. Most seedlings were not able to germinate because of the damage caused by the pathogen. *Rhizoctonia solani* caused root rot and reddish-brown sunken lesions on the stem below and above the soil line (Figure 5.2).

Figure 5.2 Disease symptoms caused by *Rhizoctonia solani* on cowpea seedlings (a); and non-infected cowpea seedlings (b).

Table 5.2 Percentage emergence and diseased plants, plant height, and root and shoot dry mass of cowpea seed treated with thiram and Celest® XL and inoculated with *Rhizoctonia solani*

Treatments	Emergence (%)	Diseased plants (%)	Height (cm)	Dry shoot mass (g)	Dry root mass (g)
Uninoculated Control	91.0d**	3.0a	11.0c	9.5c	2.40d
Inoculated Control	12.0a	77.0d	4.0a	3.3a	0.52a
Thiram	41.5c	54.5bc	5.0ab	4.6a	0.93b
*Celest® XL 1x	32.0bc	61.5c	6.2ab	5.7ab	0.84ab
1.25x	38.0bc	45.0b	7.0b	4.7a	1.06b
2x	40.2c	44.0b	7.5b	4.73a	1.32c
LSD	8.1	9.5	2.9	1.9	0.4

*Celest® XL lx =100ml/100kg seed, Celest® XL 1.25x =125ml/100kg seed and Celest® XL 2x = 200ml/100kg seed

**Each value is a mean of 4 replicates of 25 seeds. Values within a column not followed by the same letter are significantly different from each other ($P \leq 0.05$) according to the Student's t-test.

5.4.3 *Fusarium solani*

The results of percentage emergence and diseased plants, plant height, and root and shoot dry mass is given in Table 5.3. Percentage emergence was increased by all treatments. Celest® XL at twice the recommended rate was not significantly different from the uninoculated control. All treatments, except Celest® XL at 1x the recommended rate significantly reduced the disease incidence on cowpea seedlings, but none of the treatments was significantly lower than the uninoculated control. Plant height was increased by all treatments when compared to the inoculated control, even though they were significantly lower than the uninoculated control. Dry shoot mass was significantly increased by all the treatments when compared to inoculated control. They were not significantly different from the uninoculated control, except for Celest® XL at 2x times the recommended rate which was significantly higher. Only Celest® XL at 2x the recommended rate and thiram were able to significantly increase dry root mass when compared to inoculated control. They did not differ significantly from the uninoculated control (Table 5.3).

5.4.3.1 Symptoms

During harvesting small brown lesions and root rot was observed on seedlings and roots of *Fusarium solani*, infected seedlings. There was also a reddish discolouration over the entire below ground stem and root system. Soft, dark brown or black cankers developed on the stem nodes and these often girdled the stem during disease development (Figure 5.3).

Figure 5.3 Disease symptoms caused by *Fusarium solani* on cowpea seedlings (a); and non-infected cowpea seedlings (b).

Table 5.3 Percentage emergence and diseased plants, plant height, and root and shoot dry mass of cowpea seed treated with thiram and Celest® XL and inoculated with *Fusarium solani*

Treatments	Emergence (%)	Diseased plants (%)	Height (cm)	Dry shoot mass (g)	Dry root mass (g)
Uninoculated Control	91.0c**	2.0a	10.75c	10.6b	2.40c
Inoculated Control	33.0a	42.0d	4.5a	4.6a	1.25a
Thiram	80.0b	29.5bc	8.5b	12.2b	2.15bc
*Celest® XL 1x	72.8b	33.3cd	9.5b	9.9b	1.60ab
1.25x	76.3b	22.5b	9.0b	11.1b	1.60ab
2x	80.5bc	25.0bc	9.0b	12.8c	1.88bc
LSD	11.2	8.5	1.7	1.2	0.56

Celest® XL lx =100ml/100kg seed, Celest® XL 1.25x =125ml/100kg seed and Celest® XL 2x = 200ml/100kg seed

**Each value is a mean of 4 replicates of 25 seeds. Values within a column not followed by the same letter are significantly different from each other ($P \leq 0.05$) according to the Student's t-test.

5.5 Discussion

Treating seeds with thiram and Celest® XL proved to be effective against *R. solani, F. solani* and *P. ultimum* on cowpea under greenhouse conditions in this study. The active ingredients in Celest® XL are fludioxonil and mefenoxam. Mefenoxam (metalaxyl) is registered to control *Pythium* and *Phytophthora* spp. (www.syngenta.com b). It is active mainly against Oomycetes but also against other classes of fungi (Malvick & Grunden, 2004). The results from this study concur with the finding by Butcher & Pedersen (2004) who indicated that mefenoxam reduced disease incidence of both *P. ultimum* and *R. solani* of soybean. Similar findings were reported by Martinez-Espinoza *et al*. (2004) where the authors indicated that mefenoxam was effective against *R. solani* on ornamentals. Chase (1999) and McGovern *et al*. (2001) also found mefenoxam to be effective in reducing disease incidence caused by *Fusarium* spp.

The ability of Celest® XL to control *P. ultimum* was also echoed by Mathre *et al*. (1993) where the author indicated that, under field conditions treating seed prior to planting with metalaxyl, reduced damping-off caused by *P. ultimum* and improved seedling emergence on maize. Similarly, Wayne *et al.* (2003) indicated that addition of metalaxyl to captan as a seed treatment significantly reduced *Pythium* compared to a seed treatment with captan only on maize. Suet (1990) found that seed treatment with metalaxyl effectively controlled *Pythium* damping-off of pea (*Pisum sativum* L.). A seed treatment with a mixture of metalaxyl and cloroneb (a systemic fungicide highly fungistatic to *R. solani*) was successfully used to control the damping-off disease complex of cotton caused by *P. ultimum* and *R. solani* (Hancock, 2004).

Celest® XL also contains fludioxonil. This fungicide is registered to control *Rhizoctonia* spp., *Pythium* spp. and *Fusarium* spp.(www.syngenta.com b). In the current study Celest® XL was highly effective in reducing the disease incidence. This finding concurs with Butcher & Pedersen (2004) who found fludioxonil to be effective against *R. solani* on soybean. The results also agree with Kojima *et al.* (2004) who reported that in the presence of fludioxonil, *R. solani, F. solani* and *P. ultimum* were not able to infect the

host due to the failure of the pathogens to develop appressoria. The positive findings of the current study also concur with Kataria *et al.* (2002) who indicated that fludioxonil was effective in controlling *R. solani.*

At a higher concentration of Celest® XL (2x the recommended rate), all the plants showed fewer symptoms of the disease. Smith *et al*. (1999) reported that at 1.5x the recommended rate, fludioxonil can effectively control *C. dematium* of cowpea. Similar results were reported by Van den Berg *et al*. (2001) when working with *Alternaria* diseases of cowpea. All concentrations of Celest® XL evaluated in this study controlled *Rhizoctonia*.

Thiram was able to control all three pathogens because it contains dithiocarbarmates, which are toxic to fungi as they have metabolites of the isothiocyates radial (Agrios, 2005). The results of the study agrees with those found by Bhardwaj & Shrestha (2003) where they concluded that treating soybean seeds with thiram controlled many seedborne diseases Payne & Williams (1990) also reported that improved seed establishment and reduced post-emergence losses caused by *Pythium* species on sugarbeet (*Beta vulgaris* L.) could be achieved by seed treatment with thiram and hymexazole.

Even though seedlings showed vigorous symptoms caused by all the pathogens at 1x and 1.25x the recommended rate, Celest® XL was able to control these pathogens. Symptoms were reduced as the concentration of Celest® XL increased especially in *P. ultimum* and *F. solani* inoculated medium. The pathogen strain of *R. solani* was very aggressive and this resulted in poor germination and severe symptoms on germinated seedlings.

Seed treatment with Celest® XL proved to very effective in this study. *Rhizoctonia solani, F. solani* and *P. ultimum*, which contribute to yield reduction of cowpea, can be better controlled if this fungicide is registered as a seed treatment on cowpea. Celest® XL also has an ability to improve germination by reducing the effect of storage fungi. This contributes towards the livelihood of less privileged farmers who store their seed under poor conditions. The method of seed treatment is simple and less time consuming than

other seed treatment methods. Seed treatment can be done a day before planting. Celest® XL is not phytotoxic even at 2x the recommended rate. For areas known to be highly infested with *R. solani* a higher dosage of 2x the recommended rate would work effectively. The overall conclusion from this study is that Celest® XL should be tested further in the field in order to register it as a seed treatment fungicide for cowpea.

CHAPTER 6

GENERAL DISCUSSION

The results of this study showed that thiram was able to inhibit *P. ultimum* mycelial growth *in vitro*. In the greenhouse trials thiram reduced disease incidence of *P. ultimum*, when compared to the inoculated control but there was a higher percentage of diseased seedlings than the uninoculated control. Thiram is a protectant fungicide that protects the seedcoat and seedling at germination. After seedling emergence the pathogen continues to attack the seedling. The high percentage of infected seed may also be due to *P. ultimum* being a fast growing pathogen. This was observed in the *in vitro* test where in the 6th day the pathogen had already grown up to 60mm in the Petri dishes.

Celest® XL effectively inhibited mycelial growth of *P. ultimum*. The pathogen was completely inhibited at 2x the recommended rate. This may have been due to the active ingredients in the fungicide that has both a protective and curative nature. In the greenhouse Celest® XL effectively reduced infection by *P. ultimum* when compared to inoculated control but not to the same level as the uninoculated control. Seeds were protected by the fludioxonil (protectant fungicide) component of Celest® XL. During germination and after emergence mefenoxam, a systemic fungicide, continued the protection against the pathogen. *In vitro* and *in vivo* tests in this study prove that *P. ultimum* of cowpea can be effectively controlled using Celest® XL.

Thiram proved to be effective against *F. solani* throughout the *in vitro* test. A similar trend was observed in the greenhouse trials. This is due to thiram being a derivative of the dithiocarbamates, metabolites of the isothiocyates (Agrios, 2005) As a result of this action thiram is registered as a seed treatment fungicide on beans (*Phaseolus vulgaris* L.). Although thiram reduced infection by *F. solani*, where only 29% of the germinated plants were diseased. The higher percentage of diseased plants may be due to seedlings being exposed to *F. solani* that had colonized the growing medium. As the seedlings germinated they were exposed to the pathogen since thiram only works as a preventative fungicide on seedcoats.

Celest® XL was effective in inhibiting mycelial growth of *F. solani* at all concentrations in the *in vitro* assay. The same observations were seen in the greenhouse. The higher concentration of the fungicide resulted in a better protection of the seedlings. The current study shows that Celest® XL was effective against *F. solani.*

Thiram was able to control *R. solani* in both *in vitro* and *in vivo* trials to a large extent. A reduction in diseased seedlings (54%) was observed when compared to the 77% diseased seedlings of the inoculated control but did not reach the level of the uninoculated control (3%). The pathogen culture used in the trials was extremely aggressive and thiram may have been less effective.

In the current study Celest® XL was also effective against *R. solani*. This fungicide significantly inhibited mycelial growth of the pathogen *in vitro*. This shows that the active ingredients (fludioxonil and mefenoxam) were able to control the pathogen. The percentage diseased seedling was higher when compared to the uninoculated control but significantly lower than the inoculated control. Seedlings treated with Celest® XL at 2x the recommended rate showed fewer symptoms when compared to those treated by Celest® XL at 1x and 1.25x the recommended rate.

In the germination experiment, treating cowpea seed with thiram resulted in improved germination and shoot and root length, when compared to the control. The improved germination and seedling growth was due to thiram controlling the storage and saprophytic fungi associated with the seed, which are known to reduce germination of untreated seeds. Percentage diseased seed was greatly reduced by eliminating storage fungi and saprophytic fungi. Thus, thiram as a broad-spectrum fungicide was able to enhance seedling germination and performance in the current study. In the greenhouse thiram was unable to improve plant height and dry shoot and root mass when compared to uninoculated control due to lack of seedling protection after emergence.

Celest® XL improved germination, shoot and root length of the cowpea seedlings in the germination studies, as it also eliminated storage fungi. This study showed that Celest® XL improved seed germination as treated seeds germinated far better than the control. Germination performance was enhanced by treating seeds with Celest® XL, due to the combination of the active ingredients (fludioxonil and mefenoxam), which eliminated saprophytic microorganisms and storage fungi.

In general, there was a reduction in emergence and plant height in the greenhouse, which may be due to the seed treatment method. The seed is treated with the particular fungicide and then dried before planting the next day, this may trigger germination and, drying may disrupt some already expanding cells in the seed (Bewley & Black, 1994). Treating seeds with Celest® XL reduced both the number of abnormal and diseased seedlings in the germination test. No phytotoxic effect was observed even at 2x the recommended rate. It can be concluded that Celest® XL improved cowpea seed performance and eliminated storage and saprophytic fungi, which result in poor seed germination, abnormal and diseased seedlings.

Percentage germination was lower in the germination test when compared to emergence in the greenhouse. The greatly improved emergence of the uninoculated control in the greenhouse may have been due to the reduction of storage and saprophytic fungi on the seeds by the general microflora present in the growing medium. The optimal condition for the storage fungi and the saprophytes and the lack of competition by other microorganisms played a role in the lower germination in the laboratory.

Root and shoot length of the control in the germination test were lower when compared to those of the treated seed possible due to the negative effect of the presence of saprophytes and storage fungi. However in the greenhouse root and shoot mass of the uninoculated control was higher when compared to those treated with the fungicide. This may be due to the balance in microflora in the greenhouse-growing medium as compared to the laboratory. Root and shoot length of the treatments was significantly higher in the germination test when compared to the control. In the greenhouse only seedlings infected

with *R. solani* had a significantly lower root and shoot mass when compared to the uninoculated control.

The observations in this study show that Celest® XL and thiram were effective in controlling the three cowpea seedling diseases. The promising results show the ability of the fungicides to control the three pathogens. This was observed in the *in vitro* test, germination test and greenhouse trials.

Recommendations

Sufficient data is provided in this study to suggest that further testing of the treatments under field conditions is required, where residual effects of the fungicide on both plant and soil can also be tested. However, the financial viability of using these fungicides as seed treatments of cowpea need to be determined to ultimately allow the fungicides to be registered as seed treatments for cowpea in South Africa.

REFERENCES

ABBASSI, Y.O., ODEBIYI, J.A. & TAMO, M. 2004. Novel use of fish emulsion as disease control product: Management of soilborne and foliar plant pathogen. *Phytopathology* 94: 543-551.

ADANDONON, A., AVELING, T.A.S., LABUSCHAGNE, N. & AHOHUENDO, B.C. 2001. *Pythium/Rhizoctonia* complex causing damping-off of cowpea in South Africa. *African Plant Protection* 7: 111-113.

ADANDONON, A., AVELING, T.A.S., LABUSCHAGNE, N. & AHOHUENDO, B.C. 2003. Epidemiology and biological control of the causal agent of damping-off and stem rot of cowpea in the Ouémé valley, Bénin. *Agronomical Science* 6: 1-2.

ADANDONON, A., AVELING, T.A.S. & TAMO, M. 2004. Occurrence and distribution of cowpea damping-off and stem rot and association with fungi in Benin. *Agricultural Science* 142: 561-566.

ADANDONON, A., AVELING, T.A.S. & TAMO, M. 2005. Occurrence and distribution of cowpea damping-off and stem rot associated with fungi in Benin. *Australian Journal of Plant Pathology* 67: 234-241.

AGARWAL, S.C. & NEMA, S. 1994. Effect of carbendazim on *Macrophomina* leaf spot of black and green gram. *Indian Journal of Plant Protection* 17: 147-149.

AGARWAL, V.K. & SINCLAIR, J.B. 1996. Effect of fungicidal seed treatment on emergence, nodulation and grain yield of soybean in Marathwada. *Journal of Maharashtra Agricultural University* 4: 112-116.

AGRIOS, G.N. 2005. Plant Pathology. 5th Ed., Academic Press, New York. pp 339-341.

ALABI, O., EMECHEBE, A. & TYAGI, P.D. 1986. Laboratory and greenhouse evaluation of fungicides for the control of brown blotch of cowpea. *Samaru Journal of Agricultural Research* 4: 25-33.

ALABI, O. & EMECHEBE, A.M. 1990. Field evaluation of seed treatment and leaf spray fungicides for the control of cowpea brown blotch induced by *Colletotrichum capsici. Samaru Journal of Agricultural Research* 7: 151-158.

ALEXANDRA, W., VANDERWILK, F. & VENBEREK, M. 2002. Faba bean necrotic yellow mosaic virus on cowpea. *Virology* 286: 976-984.

ALLEN, D.J. 1983. The Pathology of Tropical Food Legumes (Disease Resistance in Cowpea Improvements). John Wiley and Sons, Chichester. pp 188-228.

ALLEN, D.J. 2004. The pathology of tropical food legumes (disease resistance in cowpea improvements). John Wiley and Sons, Chichester. pp 188-228.

AMADIOHA, A.C. 2003. Evaluation of some plant leaf extracts against *Colletotrichum lindemuthianum* in cowpea. *Phytopathology and Entomology* 38: 259-265.

AMIN, K.S. 1991. Pea stem rot and its chemical control. *Indian Phytotopathology* 34: 224-225.

AMUSA, N.A. & ADEGBITE, A.A. 2003. The major economic field diseases of the humid agro-ecologies of Southwest Nigeria. *Applied Science* 4: 12-18.

ANASO, K., WOODSON, D. & FAGRO, S. 1988. Effect of thiram and captan as seed treatment on soybean yield. *Plant Disease* 45: 234-240.

ATACHI, P., DESMIOTS, M., & DURNEZ, C. 1984 Investigation of cowpea (*Vigna unguiculata* (L) Walp.) Pest in the Republic of Benin (1975-1982). Technical Report-Agriculture Research, Cotonou, Benin.

AVELING, T.A.S. & ADANDONON, A. 2000. Pre- and post-emergence damping-off of cowpea caused by *Pythium ultimum* in South Africa. *Plant Disease* 84: 922.

AVELING, T.A.S., ADANDONON, A., KRITZINGER, Q., PAKELA, Y.P., SMITH, J.E. & VAN DEN BERG, N. 2001. Research Development on Cowpea Diseases in South Africa. Biennial Plant Pathology Conference Proceedings. Australasian Plant Pathology Society, Australia.

AYKOYD, F.A., ALABI, Y.O., ODEBIYI, J.A. & TAMO, M. 1982. Cowpea in Nigeria. Proceeding of a symposium on nutritional improvement of food legume by breeding, 3-5 July, 1972, New York, NY, United Nations Protein Advisory Group. pp151-158.

BANKOLE, S.A. & ADEBANJO, A. 1996. Biocontrol of brown blotch of cowpea, caused by *Colletotrichum truncatum* with *Trichoderma viridae*. *Crop Protection* 15: 633-636.

BEWLEY, J.D. & BLACK, M. 1994. Seeds: Physiology of Development and Germination. Plenum Press, New York. Pp 445.

BHARDWAJ, L.N. & SHRESTHA, S.M. 1985. Efficacy of fungicide application in the control of stem gall of Coriander. *Agriculture, Ecosystems and Environment* 13: 319-323.

BHASKAR, A.V., RAO, K.C.S. & RAHMAN, M.A. 2005. Occurrence and management of dry corm rot disease (*Fusarium solani*) in Colocasia. *Biological Science* 21: 221-230.

BIERMAN, R. E., RIECHERS, D.E., SPRAQUE, C.L. & BOLLERO, G. 2006. Fungicide herbicide interaction in soybean (*Glycine max*). *Crop Protection* 25: 134-139.

BOYHAN, G.E., GRANBERRY, D.M. & KELLY, W.T. 1999. Southern peas. The University of Georgia College of Agricultural and Environmental Sciences Georgia, United States, pp 1-4.

BRANTNER, J.R. & WINDELS, C.E. 1998. Variability in sensitivity to metalaxyl in vitro, pathogenicity, and control of *Pythium* spp. on sugar beet. *Plant Disease* 82: 896-899.

BULGARELLI, M.A., BEUCHAT, L.R. & MCWATTERS, K.H. 1988. Microbiological quantity of cowpea paste used to prepare Nigerian "Akara''. *Food Science* 53: 442-449.

BUTCHER, E.S. & PEDERSEN, W.L. 2004. Evaluation of fludioxonil and azoxystrobin for control of ***Rhizoctonia solani*** of soybean. *Phytopathology* 94: 2345-251.

CAHILL, L. 2000. National Registration Authority for Agricultural and Veterinary Chemicals. Novartis Crop Protection, Kingston, Australia. pp 1-4.

CARISSE, O., BASSAM, S.E. & BENHAMOU, N. 2001. Effect of *Microsphaeropsis* sp. strain P1 30A on germination and production of sclerotia of ***Rhizoctonia solani*** and interaction between the antagonist and the pathogen. *Phytopathology* 91: 782-791.

CARROLL, J.E. 2004. Monoculture and Disease Epidemics. Cornell University in Cooperation with the National Association of Biology Teachers. Cornell University. pp 1-4.

CERCAUSKAS, R. 2001. *Fusarium* Stem and Fruit Rot of Greenhouse Pepper. Ministry of Agriculture and Food. Fact sheet. Government of Ontario, Canada. pp 1-8.

CESSE, N. 1995. Registration of 'Mouride' cowpea. *Crop Science* 35: 1215-1216.

CHASE, N. 1999. *Pythium* and *Phytophthora* control update. www.westernfarmservice.com 2006/03/12.

CHATTERTON, S., SUTTON, J.C. & BOLAND, G.J. 2003. *Phytophthora* and *Pythium* species associated with root rot of young beans and their control. *Soil Biology* 11: 1059-1053.

CHEN, W., SCHNEIDER, R.W. & HOY, J.W. 1992. Taxonomic and phylogenetic analysis of ten *Pythium* species using isozyme polymorphisms. *Phytopathology* 82: 1034-1044.

DAFERERA, D.J., ZIOGAS, B.N. & POLISSIOU, M.G. 2002. The effectiveness of plant essential oils on the growth of *Botrytis cinerea*, *Fusarium* sp., and *Clavibacter michinganensis* subsp. *Michiganensis*. *Crop Protection* 22: 39-42.

DAVIS, D.W., OELKE, E.A., OPLINGER, E.S., DOLL, J.D., HANSON, C.V. & PUTNAM, D.H. 1991. Cowpea. University of Wisconsin-Madison, United States. pp 1-10.

DAVIS, R.M., NUNEZ, J.J. & SUBBAOA, K.V. 1997. Benefits of cotton seed treatments for the control of seedling diseases in relation to inoculum density of *Pythium* spp. and *Rhizoctonia* spp. *Plant Disease* 81: 766-768.

DAVIS, D.W., OELKE, E.A., OPLINGER, E.S., DOLL, J.D., HANSON, C.V. & PUTNAM, D.H. 2004. Cowpea. University of Wisconsin-Madison, United States. pp 1-10.

DEVRIES, J. & TOENNIESSEN, G. 2001. Securing the Harvest, Biotechnology, Breeding and Seed System for African Crops. CABI Publishing, New York, USA. pp 32-36.

DORRANCE, A.E., LIPPS, P.E. & MILLS, D.R. 2001. *Rhizoctonia* Damping-off and Stem Rot of Soybeans. Extension Fact-sheet. Plant Pathology, Columbus, Ohio State University. pp 1-2.

DUFFY, B. 2000. Combination of pencycuron and *Pseudomonas flourescens* strain 2-79 for integrated control of *Rhizoctonia solani* and take all of wheat. *Crop Protection* 18: 234-240.

DURANT, F., DAVIDS, J. & SPENSER, R. 1988. Effect of thiram as steep seed treatment on sugar beet. *Plant Disease* 72: 874-889.

EBBELS, D.L. 1993. Effect of fungicide seed treatment on plant health and germination. *Seed Science and Technology* 7: 75-81.

EDEMA, R., ADIPALA, E. & FLORINI, D.A. 1997. Influence of season and cropping system on occurrence of cowpea diseases in Uganda. *Plant Disease* 81: 465-468.

ELLIS, M. & PASCHAL, E. 1990. Transfer of technology in seed pathology of tropical legumes. *Seed Pathology* 42: 316-322.

EMECHEBE, A.M. & FLORINI, D.A. 1997. Shoot and pod diseases of cowpea induced fungi and bacteria. *Crop Science* 30: 350-358.

FARITH, A., TSAO, P.H. & MENGE, J.A. 1981. *In vitro* effects of metalaxyl on growth, sporulation, and germination of *Phytophthora parasitica* and *P. citrophthora*. *Plant Disease* 65: 651-653.

FERRIS, S. & MITCHEL, J. 1991. Population dynamics of soil microorganisms associated with fungicide dusted caladium seed piece. *Soil Biology and Biochemistry* 13: 57-63.

FISHER, D.J. & HAYES, A.L. 1982. Mode of action of the systemic fungicides Furalaxyl, Metalaxyl and Ufurace. *Biological Science* 13: 330-339.

FRANEKI, J. & BRATULI, M. 1994. Effect of dithinocarbamates as seed protectant agriculture. *Plant Disease* 71: 65-75.

FRANEKO, D. 2003. Studies on *Nelumbo lutea* (wild) pear I. Technique for axenic liquid seed culture. *Aquatic Botany* 26: 113-117.

FRAVEL, D.M., DEAHL, K.L. & STOMMEL, J.R. 2004. Compatibility of the biocontrol fungus *Fusarium oxysporum* strains CS-20 with selected fungicides. *Biological Control* 34: 165-169.

GHALES, S., SUMMER, D. & PHATEK, E.S. 1991. Stan and yield of mungbeans seeds with gel and fungicides in various tillage systems. *Crop Protection* 10: 23-27.

GLEN, H., Y-DOW, H. & SUSAN, L. 2003. Characterization of Soybean Pathogens and Disease Management. Australian Plant Pathology Society, Australia. pp 1-4.

GONCALVES, P., ANDERSON, R.L., HOFFARD, W.H., DLANDIS, T.D., SMITH, R.S. & TOKO, H.V. 1991a. *Rhizoctonia solani*. North Carolina State University, Carolina. p. 728 . a

GONCALVES, E.J., MUCHOVEJ, J.J. & MUCHIVEJ, R.M. 1991b. Effect on kind and method of seed treatment of beans seed on infection by Va- *mycorrhia*l fungus and by pathogenic fungus *Fusarium solani. Plant and Soil* 11: 41-46. b

GOWILY, A.M. & SOLIMAN, G.I. 1994. Effect of seed dressing with some fungicides and some agricultural practices on controlling broad bean roots diseases caused by *R. solani. Annals of Agriculture Science* 32: 234-240.

GREMELL, L. & HERRIDGE, F. 2004. Lime pelleting inoculated serradella seed *Ornithopus* species increase nodulation and yield. *Soil Biology and Chemistry* 36: 1289-1294.

HALL, A.E. 1997. Cowpea breeding. *Plant Breeding Review* 15: 268-274.

HAPTON, R., THOTTAPPILY, G. & ROSSEL, H. 1997. Viral diseases of cowpea and their control by resistance-conferring genes. *Experimental Agriculture* 26: 341-362.

HANCOCK, N. 2004. Evaluation of different fungicides in controlling the rhizome rot on legumes under storage conditions. *Annals of Agricultural and Biological Research* 9: 63-65.

HARIKRISHNAN, R. & YANG, X.B. 2004. Recovery of anastomosis groups of *Rhizoctonia solani* from different latitudinal positions and influence of temperatures on their growth and survival. *Plant Disease* 88: 817-823.

HARRISSON, A.R. & NELSON, S. 1999. Study of *Rhizoctonia solani* as a threat to leguminous crops. *Crop Protection* 19: 28-34.

HARRIS, A.R.& NELSON, S. 1999. Progress toward integrated control of damping-off diseases. *Microbiological Research* 154: 123-130.

HARRIS, A.R., FEDIS, G. & NELSON, S. 1999. Progress toward integrated control of damping-off diseases. *Microbiological Research* 154: 123-130.

HILL, D.S., STEIN, J.L., TORKEWITZ, R.N., MORSE, A.M., HOWELL, C.R., PACHLATKO, J.P., BECKER, O.J. & LIGON, P.N. 1994. Cloning of genes involved in the synthesis of pyrrolnitrin from *Pseudomonas fluorescence* and role of pyrrolnitrin in biological control of cotton diseases. *Crop Protection* 15: 123-127.

INGLIS, D.A., POWELSON, M.L. & DORRANCE, A. 1999. Effect of registered potato seed piece fungicides on tuber-borne *Phytophthora infestans*. *Plant Disease* 83: 229-234.

International Seed Testing Association. 2005. International rules for seed testing. *Seed Science and Technology* 27 supplement, pp 33.

JONES, K.R. 2000. Assessment of *Fusarium* head blight of wheat and barley in response to fungicide treatment. *Plant Disease* 84: 1021-1030.

JONES, R. & SAMAC, A. 1996. Biological control of fungi causing alfalfa seedling damping-off with a decrease in suppressive strain of *Streptomyces* and metalaxyl. *Biological Control* 7: 1196-204.

KASSOU, D.K., GHEHOUNOU, G., AHANCHEDE, A., AHOHUEDO, B., BOURAIMA, Y. & VAN HIS, A. 2001. Indigenous cowpea production and protection practices in Benin. *Insects Science and Its Application* 21: 123-132.

KATARIA, H.R. & DODAN, D.S. 1981.The influence of two herbicides on antifungal activiy of some soil against *Pythium butleri* and *Rhizoctonia solani* causing damping off on cowpea. *Pesticide Science* 13: 583-588.

KATARIA, M.S., MADDEN, L.V. & HOITINK, A.J. 1989. Effects of potting mix microbial carrying capacity on biological control of *Rhizoctonia* damping-off of radish and *Rhizoctonia* crown and root rot of poinsettia. *Phytopathology* 79: 1116-1124.

KATARIA, H.R. & GROVER, R.K. 1991. Comparison of fungicides for control of *Rhizoctonia solani* causing damping-off of mungbeans (*Phaseolus aureus). Annals of Appied Biology* 88: 257-263.

KATARIA, H.R., YADAV, J.P. & DROVER, R.K. 1991. Interaction of chemical fertilizer with seed dressing fungicides controlling *Rhizoctonia solani*. *Plant Pathology* 11: 641-650.

KATARIA, H.R., WILMSMEIER, B. & BUCHENANER, H. 2002. Efficacy of *Pseudomonas flourescens* strains and some modern fungicides for controlling *Rhizoctonia solani* AG4 in bean and cucumber. *Plant Pathology* 109: 384-400.

KATARIA, H. & GROVER, H. 2004. Interaction of fungicides-insecticides combinations against *Rhizoctonia solani* in vitro and soil. *Crop Protection* 23: 399-404.

KILLEBREW, F. 2001. Control of virus diseases by using the right varieties in field crop. *Field Crop* 45: 567-573.

KIRKPATRICK, F. & ROTHROCK, G. 2001. Infection of Danish seeds by *Rhizoctonia solani* Kühn. *Plant Disease* 85: 1276-1278.

KITCH, L.W., BOUKAR, O., ENDONDO, C. & MURDOCK, L. 1998. Farmer Acceptability Criteria in Breeding Cowpea. Cambridge University Press, UK. pp 24-31.

KOCH, E. 1997. Screening of rhizobacteria for antagonistic activity against *Pythium ultimum. Plant Science* 44: 353-361.

KOENNING, S. 2002 Cotton Seedling Disease. Plant Pathology Extention. College of Agriculture and Life science North Carolina State University, United State of America. pp 1-5.

KOJIMA, K., TOKANOY, V., YOSHIMA, A., TANAKA, C., KIKUCHI, T. & OKUNO, T. 2004. Fungicide activity through activation of fungal signal pathway. *Molecular Microbiology* 53: 1785-1796.

KOLLER, W., ALLAN, C.R. & KOLATTUKDY, P.E. 1982. Inhibition of cutinase and prevention of fungal penetration into plant by benomyl- a possible protective mode of action. *Crop Protection* 9: 105-113.

KRITZINGER, Q., AVELING, T.A.S. & MARASAS, W.F.O. 2002. Effect of essential plant oils on storage fungi, germination and emergence of cowpea seed. *Seed Science and Technology* 30: 609-619.

LANGYINTIUO, A.S., LOWENBURG-DEBOER, J., FAYE, M., LAMBERT, D., IBRO, G., MOUSA, B., KERGNA, A., KUSHWAHA, S., MUSA, S. & NTOUKAM, G. 2003. Cowpea supply and demand in West and Central Africa. *Field Crop Research* 82: 215-231.

LATUDE-DADA, A.O., O'CONNEL, R., NASH, J., PRING, C., LUCA, R.J. & BAILEY, J.A. 1993. Occurrence, Distribution and Pathogenicity of Cowpea root rot and stem pathogen in West Africa. *Phytophthora vigna* in soil of West Africa. Plant Disease 77: 115-1168.

LEACH, N.G. & FRANEKO, J.R. 1982. *Rhizoctonia solani*, Biology and Pathology. University of California Press. Berkeley, Los Angeles and London. pp 1-4.

LORIA, R. 1993. Vegetable Crops. *Fusarium* Dry Rot of Potato. Fact Sheet. Cooperative Extension, Cornell University, New York State. pp 726.

MALVICK, D.K. & GRUNDEN, E. 2004. Traits of soybean infecting *Phytophthora* populations from Illinois agricultural fields. *Plant Disease* 88: 1139-1145.

MANKVOLD, P.G. & O'MARA, J.K. 2002. Laboratory growth chamber evaluation of fungicide seed treatment of maize seedling blight caused by *Fusarium* species. *Plant Disease* 86: 143-150.

MARASAS, W.F.O., NELSON, O.E. & TOUSSOUN, T.A. 1984. Toxigenic *Fusarium* spp. Identity and Mycotoxicology. The Pennsylvania State University, United States of America. pp 263-265.

MARSHAL, G.M. 1977. Effect of seed treatment in preventing transmition of dwarf bunt of winter wheat to new areas. *Canadian Journal of Plant Pathology* 2: 201-204.

MARTINEZ-ESPINOZA, A.D., MUELLER, D.S. & BUCK, J.W. 2004. Efficacy of fungicides for *Rhizoctoni*a root rot control on *Cathara roseus*. *Biological Sciences* 94: 2004-2019.

MATHRE, D.E, CALLAN, N.W., JOHNSTON, R.H., JAMES, B., & SHWED, A. 1993. Factors influencing the control of *Pythium ultimum* induced seed decay by seed treatment with *Pseudomonas fluorescence* AB 254 and metalaxyl. *Crop Protection* 13: 301-107.

MAUDE, R.B. 1996. Seedborne Diseases and Their Control, Principles and Practices. CAB International, Washington. pp 136-239.

MAURICIO, C., BUENO, J., NILTON, C. & YORINORI, T. 2005. Effect of doses of fungicides and plant resistance activators on control of Rhizoctonia foliar blight of soybean and on *Rhizoctonia solani* AGL-1A *in vitro* development. *Crop Protection* 25: 848-854.

MAZZOLA, C. 1998. Effect of fludioxonil as drench fungicide for controlling soilborne diseases on apple seedling. *Annal of New York Academy of Science* 17: 406-414.

McGOVERN, R.J., ELMER, W.H., GIESER, D.M. & HARBAUGH, B.K. 2001. Biology, Epidemiology, and Integrated Management of Diseases Caused by *Fusarium* in Spotted Ornarmentals. University of Florida Department of Plant Pathology, Gainesville United State of America. pp 1-5.

McMULLEN, M. & BRADLEY, C. 2005. Field crop fungicide guide. North Dakota State University, United States of America. pp 1-6.

MILLER, C.S. 1994. Effect of dry seed treatment on soybean. *Plant Disease* 71 : 89-101.

MILLS, A.S., PLAT, W.H. & HURTA, A.R. 2004. Effect of salt compounds on mycelial growth, sporulation and germination of various potatoes pathogens. *Postharvest Biology and Technology* 34: 341-350.

MONTERMAYOR, M. 1995. Effect of different seed treatment method on cotton emergence. *Journal of Agriculture Engineering Research* 61: 129-136.

MOORMAN, G. 2004. Plant Disease Facts. Penn State University Cooperative Extension. USA. pp 1-5.

MOUBASHER, A.H., ABDEL-KADER, M.I. & ABDEL-MALLER, A.Y. 1986. Effect of dithane M-45 on cellulose- decomposing fungi in Egyptian soil. *Soil Biology* 8: 564-570.

NAKAWUKA, C.K., MATHUR, S.B. & ADIPALA, E. 1997. Seedborne fungi and seed health of cowpea. *Plant Disease* 81:1099-1104

NELSON, P.E., TOUSSOUN, T.A. & COOK, R.J. 1981. *Fusarium*: Diseases, Biology, and Taxonomy. The Pennsylvania State University Press, USA. pp 1-4.

NENE, Y. & THAPLIYAL, P. 1993. Fungicides in plant disease control. *Plant Science* 67: 789-794.

NG, O. 1995. Cowpea. Evolution of Crop Plants, 2nd ed. Longman, Harlow, UK, pp 326-332.

NISHIJIMA, W. 1993. *Fusarium solani*. Crop Knowledge Master. University of Hawaii, Hilo. pp 1-2.

OLOWE, T., DINA, S.O., OLADIRAN, A.O. & OLUNUGA, B.A. 2003. The control of weed, pests and diseases complexes in cowpea (*Vigna unguiculata* (L.) Walp) by the application of pesticides singly and in combination. *Crop Protection* 22: 222-225.

PAULITZ, T.C. 1991. Effects of *Pseudomonas putida* on the stimulation of *Pythium ultimum* by seed volatiles of pea and soybean. *Phytopathology* 81: 1282-1287.

PAULITZ, T.C. & ADAMS, K. 2003. Composition and distribution of *Pythium* communities in wheat fields in Eastern Washington State. *Phytopathology* 93: 867-873.

PAYNE, A. & WILLIAMS, G. 1990. Hymexazol treatment of sugar beets seed to control seedling diseases caused by *Pythium* spp. *Crop Protection* 9: 371-377.

PENDULOSI, S. 1997. Cowpea investment in African agriculture research. *Plant Disease* 81: 456-460.

PENDULOSI, S., PALTI, J. & ROTEM, J. 1997. Cultural Practice for the Control of Crop Diseases. In: Commonwealth Mycological Institute (CMI), Plant Pathologist Pocketbook 2nd ed., Kew, UK, CMI, pp 183-195.

PETERS, R.D., STURZ, A.V., MATHESON, B.G., ARSENAULT, W.J. & MALONE, A. 2001. Metalaxyl sensitivity of isolates of Phytophthora erythroseptica in Prince Edward Island. *Plant Pathology* 50: 302-309.

PHILLIPS, R.D., KAY, H., MCWATTERS, M.S., CHNNAM, Y.N., LARRY, R., BEUCHAT, S.S., SAKYI-DAWSON, E., NGODDY, P., NNAYELUGO, D. & ENWEREJ, C. 2003. Utilization of cowpea for human consumption. *Field Crop Research* 82: 192-213.

POUWELS, J., CARETTE, J., VAN LET, K. & JOAN, K. 2001. Effect of cowpea mosaic virus on host cell processes. *Plant Pathology* 23: 306-315.

QUASS, C.F. 1995. Production of cowpeas Vegetable and Ornamental Plant Institute, Agriculture Research Council. pp 1-9.

RAMUSI, M.T. 2006. Biological and chemical control of fungal diseases of cowpea. MSc thesis, University of Pretoria. Pretoria. South Africa. pp 21-24.

READ, P. & HIDE, G. 1995. Effect of fungicides on the growth and conidial germination of *Colletotrichum coccodes* and on the development of black dot disease of potatoes. *Annals of Applied Biology* 126: 437-447.

RENNIE, W.J. 1993. The need for cereal seed treatment in UK in the post mercury era. *Pesticide Outlook* 4: 29-34.

ROY, K. 1982. Seedling diseases caused in soybean by species of *Colletotrichum* and *Glomerella*. *Phytopathology* 72: 1093-1096.

RICHARDSON, G. 1981. Effect of different fungicides on *Rhizoctonia solani* of peas. *Plant Disease* 56: 231-239.

RUPPEL, E. & BAKER, R. 1998. Field-testing of *Trichoderma harzianum* and captan as biocontrol agent of seedling diseases in several crops and *Rhizoctonia* root rot of sugar beet. *Plant Disease* 82: 399-402.

SAETTLER, A.F. 1998. Bacterial diseases and their impact on yield of cowpeas and beans. *Plant Disease* 82: 247-256.

SCOT, J.M. 1989. Seed coatings and treatment and their effect on plant establishment. *Annals of Applied Biology* 119: 44-57.

SHERF, A.F. 1997.Control of bacterial diseases on legumes. *Field Crop* 63: 589-596.

SINCLAIR, J.B. 1993. Control of seedborne pathogens and diseases of soybean seeds and seedling. *Pesticide Science* 37: 15-21.

SINGH, R.S & ALLEN, D.J. 1997. Insects Pest on Cowpea. Manual 2.IITA, IBANDA, NIGERIA.

SINGH, R.S. & RACHIE, K.O. 1985. Cowpea research production and utilization. John Wiley & Sons. Chichester. New York. pp 276-287.

SINGH, B.B., RAJ, .M R., DASHIEL, K.E. & JACKAL, L.E.N. 1997. Advance cowpea research. Co-publication of Intenational Institute of Tropical Agriculture (IITA) and Japan International Research Center for Agriculture Sciences. IITA, Ibadan and Nigeria. Devon, UK.

SINGH, S.R. & EAGLESFIELD, R.J. 2000. Cowpea Pest and Diseases. International Institute of Tropical Agriculture, Ibadan. pp 260-263.

SINHA, O.K. & KHARE, M.N. 1977. Site of infection and further development of *Micropomina phaseolina* and *Fusarium equiseti* in naturally infected cowpea seeds. Seed *Science Technology* 5: 721-725.

SITHOLE-NIANG, I. 2000. Cowpea Improvement Through Genetic Engineering. Project proposal. University of Zimbabwe, Harare. pp123-124.

SMILEY, F. & CRAVEN, G. 1994. Effect of benomyl and iprodione on controlling *Fusarium solani* on turf grass. *Plant Disease* 45: 987-994.

SMITH, J.E. 1997. *Colletotrichum dematium* on Stems of Cowpea. MSc. Thesis, University of Pretoria. Pretoria, South Africa. pp 40-67.

SMITH, J.E., KORSTEN, L. & AVELING. T. 1999. Evaluation of seed treatment for reducing *Colletotrichum dematium* on cowpea seed. *Seed Science and Technology* 27: 591-598.

SONG, W., ZHOU, L., YANG, C., ZANG, L. & LIN, X. 2003. Tomato *Fusarium* wilt and its chemical control strategy in hydroponics system. *Crop Protection* 23: 243-247.

STENSVAND, A. 1998. Evaluation of four new fungicides against brown rot and grey mould on sweet cherries *(Prunus sativum* L.). *Test of Agrochemicals and Cultivars* 19: 70-71.

STENSVAND, A & BORVE, J. 1999. Effect of Different fungicides on sweet cherries *(Prunus sativum L*) on *Fusarium* spp. *Test of Agrochemicals and Cultivars* 20: 14-15.

STEAD, D.E. 1992. Technique for detecting and identifying plant pathogenic bacteria. *Plant Disease* 70: 291-294.

SUET, D.L. 1990. The threat of accelerated degradation of pesticides - myth or reality. *Indian Plant Disease* 73: 245-254.

SUMMERFIELD, R.J. & ROBERTS, E.T. 1985. Grain legume crops. Collins, London. pp 189-203.

SYNGENTA production fact sheet. Http://www.syngenta.com/en/ products-service/fact sheet /maxim_window.html18/05.2005 b.

TAYLOR, A.G. & HARMAN, G.E. 1990. Concept and technologies of selected seed treatments. *Annual Review of Phytopathology* 28: 321-327.

TINDAL, H.D. 1983. Vegetable in Tropics. Macmillan Press, London. pp 1-5.

TSROR, L., HAZANOVSKY, M., BEN DAVIDS, T. & DORI, I. 2005. Control of *Pythium myriotylum* on growth of soybean. *Plant Disease* 89: 150-154.

VALENZUELA, H. & SMITH, J. 2002. Cowpea. College of Tropical Agriculture and Human Resources. University of Hawaii at Monoa, Honolulu, Hawaii. pp 1-4.

VAN DEN BERG, N., AVELING, T.A.S. & VENTER, S.L. 2001. Evaluation of six fungicides for reducing *Alternaria cassiae* on cowpea seed. *Crop Protection* 21: 501-505.

WAIN, R.L. & CARTER, G.A. 1977. Seed treatment using metalaxyl on onion. *Journal of Agriculture Research* 37: 620-622.

WANG, H., CHANG, K., HWANG, F., TURNBULL, D. & HAWARD, J. 2005. *Fusarium* root rot of coneflower seedling and integrated control using *Trichoderma* and fungicides. *Biological Control* 50: 317-329.

WAYNE, P., KLINE, D. & MULLER, S. 2003. Influence of metalaxyl fungicide seed treatment on soybean to control *Pythium* under no tillage condition. *Crop Protection* 22: 647-652.

WHANG, B.K. & KIM, B.S. 1995. *In-vivo* efficacy and *in-vitro* activity of tubercidin, an antibiotic nucleoside for control of *Phytophthora capsici* blight in *Capsicum annuum*. *Pesticide Science* 44: 255-260.

WHITEHEAD, R. 1995. Seed treatment with antagonists and chemicals to control *Alternaria brassicicola*. *Seed Science and Technology* 12: 851-862.

WORTH, T. & HANCE, G. 1991. Chemical control of seedling diseases of cotton. *Indian Crop Protection* 47: 374-382.

WRATHER, J.A., ANDERSON, T.R., ARSYARD, D.M.& YORINORI, J.T. 1997. Soybean disease loss estimate for the top ten soybean producing countries in 1994. *Plant Disease* 81:107-110.

www.fao.org.com/ agric/report accessed 2006 /10/11

www.syngenta.com/en/productsservices/celest accessed 2005/10/12. a

www.syngenta.com/ Fact sheet accessed 2006/02/08.b

WYDRA, K. & SIKIRO, R. 1997. Development of Cultural on Control Practices for Cowpea Bacteria blight: Mixed cropping and influence of the planting date. Plant Health Management Division, IITA Annual report, 1997. IITA, Ibadan, Nigeria. pp156-158.

XUE, A.G. 2003. Biological control of pathogens causing root rot complex in field pea using *Clonostachys rosea* strain ACM941. *Plant Science* 93: 329-335.

ZANG, L.E., SHETTY, K.K., FORSTER, B. & WATRIN, G. 2001. A low use rate of seed treatment for controlling *Fusarium* on corn and potato. Crop Protection Council Symposium Proceedings no 76 Belfry Resort Hotel, Wishaw, North Warwickshire, UK. pp105-110.

ZOHRI, A. & GAWARD, E.I. 1992. Cowpea research, production and mycotoxin of cowpea cultivars. *Korean Mycology* 20: 252-258.